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THE CYTOLOGICAL EXPRESSION OF CHANGES IN GENE ALIGNMENT PRODUCED BY X-RAYS IN DROSOPHILA

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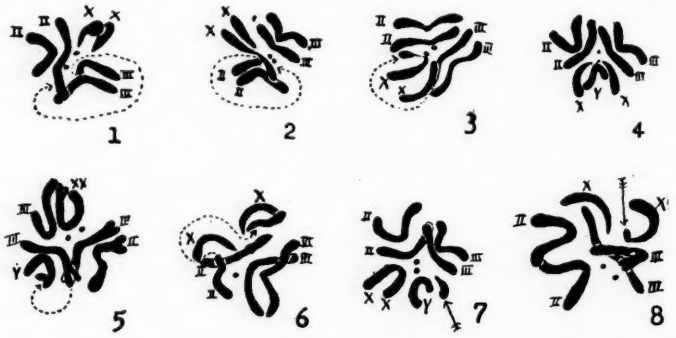
IT has been known for many years that chromosomes exposed to X-rays or radium exhibit a tendency to fragment, and also to clump or adhere together, though it has not been known whether unions of an intimate and permanent nature, and therefore of genetic significance, are thereby formed in cells which can continue to live and multiply. In X-ray work upon germ cells of *Drosophila melanogaster,* Muller and Altenburg (1928, Anat. Rec. 41: 100) have found that changes in the linkage maps are very frequent and that a section of a group of genes (chromosome) often becomes permanently attached to a non-homologous gene-group, producing a so-called "translocation," or to the same or a homologous gene-group at a different point from before, with or without the loss of another section of the gene-group, thus producing an "inversion," "duplication" or "deletion" or a combination of these, as the case may be. A large number of these genetic variants have now been isolated, and during the past year various specimens of these have been subjected to genetic and cytologic analysis at this laboratory. The purpose of this paper is to present some of the more striking results of these studies, especially the cytological, and to call attention to some of the bearings of these results. It is planned to give later, in a series of papers, the detailed genetic evidence together with the cytological findings.

We have found, through genetic tests, that all three of the major linkage groups—the X, the second and the

third—may undergo breakage following treatment with X-rays, though breakage of the Y (reported by Stern in untreated material) has not yet been looked for by us. And one of the fragments resulting may become attached to any of the other linkage groups, including the fourth and the Y.

Translocations involving groups II and III have been the most numerous, either group being the donor. The cytological picture of the most striking case of this type which we have examined so far is shown in Fig. 1 ("Star-Curly translocation"). Here one of the third chromosomes has been broken a little to one side of the main bend, or point of spindle fiber attachment, and the smaller fragment is attached to the end of a second



chromosome. Genetically the break has occurred at a little "to the left" of the center of the linkage map, half way between the genes for scarlet and pink (though this point has sometimes been supposed to be the center of the chromosome). Thus, all the genes "to the left" of this point on the map are here found to be completely linked in the male to the genes in the second linkage group, while those "to the right" still undergo recombination with the latter. In other translocations we have found shorter pieces of these chromosomes involved. In several instances the piece has been too short, physically, for the loss or gain to have been demonstrable with certainty, although a considerable number of units of a "sparse" region of the map (a region sparsely dotted with known genes) was involved, thus illustrating that a

unit in a "sparse" region is not physically as long as in another region.

A number of translocations have been found in which, as genetically determined, the donor group has been II or III and the recipient group I (the X). Thus in the case shown in Fig. 2 ("Curly to X translocation a," of Altenburg), the translocated section, belonging to group II, is linked genetically with the "right hand" (the spindle fiber) end of group I, and, as the cytological view shows, one of the second chromosomes has been broken very close to the point of spindle fiber attachment and the whole arm has joined to the X chromosome at its proximal (spindle fiber) end, producing a large V-shaped element. Superficially the chromosome picture here gives the appearance of a normal complex in which the usual arrangement of the elements is altered, because there are still two pairs of large V-like chromosomes and two rod-like elements. However, in the large number of cells which we have studied there is a marked tendency for the X-chromosome arm of the newly constructed V to lie next to the free X chromosome, and similarly the autosomal arm of this V, and the rod-like piece with which it had previously been connected, both tend to assume their normal positions next to the unbroken homolog. Another case is shown in Fig. 3 ("Dichaete to X translocation" of Altenburg). Here a small piece of chromosome III has become attached to the spindle fiber end of an X chromosome.

We have various cases in which a fragment of one of the large autosomes has become attached to the Y chromosome. In the case illustrated in Fig. 4 (III to Y translocation 2) the genetic evidence shows that a considerable number of units from the right-hand end—a somewhat sparse region—has become attached to the Y. The cytological picture, however, gives no distinct evidence either of a loss from the third or a gain by the Y chromosome, although the figures examined are exceptionally good. Either the piece concerned is physically much smaller than the number of units would indicate, due to a high frequency of crossing over in this region, or else a frag-

ment of the Y has undergone interchange, becoming simultaneously attached to the third chromosome—a matter difficult to determine genetically in the case of the relatively functionless Y chromosome. In Fig. 5, on the other hand, a case is shown (III to Y translocation 1) in which a small piece of the third chromosome is attached to the short arm of the Y, producing a small V-shaped element (in addition to the large attached X's, which were introduced by crossing with the yellow attached-X stock).

In a number of cases analyzed genetically a part of the second or third linkage group has become attached to the fourth. One of these cases, involving group II, has been analyzed cytologically also (Fig. 6). Here it can be seen that the second chromosome has broken a little beyond the point of spindle fiber attachment and the fragment appears to be free. There is, however, only one chromosome IV present in a considerable number of critical figures studied, although two IV's are present genetically; hence we conclude that the fragment is really joined to one of the IV's, having thus acquired the spindle fiber necessary for its continued existence. In one or two cells a small knob appears on the proximal end of the fragment; this probably represents the attached fourth chromosome. In the ovaries of just one fly examined two fourth chromosomes were consistently present, with the fragment lying close by; this agrees with the genetic finding that this translocation results in occasional non-disjunction of the fourth linkage group.

In numerous instances following irradiation flies have been found which gave genetic evidence for the presence of an extra portion of group I in addition to the entire X or X's which they possessed. The extra piece usually behaved as a separate element, but in one case showed linkage to group III. On genetic analysis these fragments are found to differ from one another in size, and to be made up of a block of genes from the "left hand" or free end of the map of group I, joined in some instances at least to a small piece of the right hand, or spindle fiber end, with the whole intervening block of

genes missing. The sex-differentiating gene or genes are absent in the cases of this kind thus far found. A typical chromosome complex in which this "deleted" sex chromosome lies free is shown in Fig. 7 ("X deletion 2"). Here the other X's of the female were from an attached-X race. In flies of this same stock, by means of a second irradiation, we have gotten this fragment joined to the spindle fiber end of a single X, the latter having resulted from a simultaneous breaking apart of these attached X's. The case was first analyzed genetically as being of this character, and again, as shown in Fig. 8 ("X duplication 1"), the cytological observations conform with the new linkages found. It may also be mentioned that the fragment of an X behaving as attached to group III has been found cytologically to be attached to chromosome III.

In all the figures the large autosomes have been numbered because the translocations studied have allowed us to reach definite conclusions for the first time regarding the relative appearances of chromosomes II and III under the microscope. Chromosome II contains more chromatin than III and typically appears as longer, or, when relatively condensed, as thicker. However, it is not always possible to distinguish between the two because these studies also show that the rate at which the chromosomes of different pairs in a given cell contract is subject to a good deal of individual variation. The positive identification of the second as the largest of the autosomes is in agreement with the recent tentative expression by Bridges on the same subject (Morgan, Sturtevant and Bridges, 1929, Carnegie Inst. Yearbook No. 27, pp. 330–335).

In all the cases so far found in which the cytological picture has disclosed that a section of a chromosome has become broken off from its original connection, and either lost or reattached elsewhere, and in which at the same time a genetic analysis has been made, it has been found not only that certain genes have lost their original linkage relations with others in one of the linkage groups, in fact, in that particular linkage group which is ordi-

narily taken as corresponding with the chromosome here seen to be broken, but also that the genes involved form a "block," that is, *they constitute a coherent section of the linkage map.* Thus, in the "Star-Curly translocation" of Fig. 1, all the genes investigated that lie to the left of a given locus ("46") in group III behave as though separated from all the genes to the right of this point (although those to the left are still linked as closely as they ever were to each other, as also are those to the right). There is no escape, then, from the conclusion that the previously constructed genetic map represented the genes as correctly distributed with respect to the two sides of the breakage point, in that all those which had been shown by the map as lying to the left of this point were actually on one side of it, and all those shown on the right of the point were really on the other side of it—regardless (for the time being) of whether or not the more detailed arrangement of the genes on a given side with respect to each other was also correct. If, however, the more detailed arrangement, on each side, were not also correct physically, it would have been a strange phenomenon that there should have been any such point at all, with respect to which the map distribution of genes, to right and left of it, should have been physically correct, and still stranger that the breakage should have occurred at exactly this point.

Since, now, breakages at various points have been found to occur, and the same phenomenon as above described has been encountered, wherever genetic analysis has been made, with regard to each of these *sample points,* we are driven to conclude that the *entire arrangement* is correct, *i.e.,* the order of genes on the linkage map is the same as their actual physical order in the chromosome. Previous genetically determined cases of small inherited deficiencies (Bridges, Mohr) and Stern's genetically determined cases of two kinds of large deficiencies involving group III in somatic cells had of course pointed in the same direction, as had also the earlier findings that in normal group interchange ("crossing over") whole blocks of the genetic map were

concerned (Sturtevant, Muller), but since in none of these cases parallel cytological evidence of an actually broken or interchanged chromosome could be obtained, direct proof that the loss or exchange of the block of genes of the map was really due to the loss or exchange of a morphological section of the chromosome was still lacking. That direct proof is now provided. In some of the present cases, too, we have the further cytogenetic parallelism that the fragment has become visibly reattached at a particular place on another chromosome —*e.g.*, the spindle fiber end of the X, as in Figs. 2, 3 and 8—and that genetically the translocated map segment is now linked most closely to that portion of the map of the other group which, on the theory of the physical validity of the map arrangement, corresponds to this point. There can, therefore, no longer be room for doubt that the arrangement (that is, the order, but not necessarily the relative distances) of the genes as given in the linkage maps is the same as their actual, physical arrangement.

In view of this, it follows too that the interchange which occurs between homologous groups of genes prior to segregation is an interchange of entire morphological sections of the homologous chromosomes, since it is well known that genetically the linkage maps then exchange entire blocks of genes (hence, as we now see, sections of chromosomes), rather than individual units. But to say this is to say that the so-called "mechanical theory of crossing over" is correct—the theory which holds that the chromosomes, in their normal process of interchange, become broken at one or a few points, and reattached to their linear sequels of the previously homologous members.

But while the order of the genes in the linkage maps thus stands confirmed, the same can not be said of the relative distances there shown. Thus, some translocations involving over thirty units of a relatively "sparse region" are not readily visible under the microscope (*e.g.*, Fig. 4), while conversely a fragment may appear of considerable size (as in Fig. 7) when it

represents only a few units genetically, when those units include a very "crowded region." This means that the scale of our maps, based on the frequency of crossing over, varies greatly from one region to another, but that these variations in the scale can be gaged through a combined genetic and cytological study of translocations, deletions, etc. From the evidence so obtained new maps can then be constructed showing not only the linear arrangement of the genes but also their actual spatial positions, or loci, in the chromosomes, and these maps will probably appear very different from our present maps.

For the further analyses of genetic and chromosomal phenomena of various kinds, induced chromatin rearrangements place in our hands a new and invaluable method of attack. Among the matters that such work may have a bearing on are questions regarding evolutionary changes in chromosome morphology and configuration, possible interspecific sterility and other forms of physiological isolation in evolution thereby resulting, the synaptic properties of chromosomes and genes, possible changes in crossing-over properties with changes in position of chromosome sections, the nature of intrachromosomal cohesions and interchromosomal adhesions (*e.g.*, can chromosomes have a branching structure?), the possible genetic effects of changes in gene contiguities, the possible relationship between some chromatin rearrangements and some supposed gene-mutations, including those of some ever-sporting genes, the mechanism of gene-manifestation as studied through phenotypic effects of induced "genic unbalance" and through dominance relations in hyper- and hypo-diploids, the tracing down of the sex-differentiators and the determination of the genetic termini of the linkage maps (by adding "markers" to them). Not least important among the uses of these chromatin changes there must also be mentioned the provision of new stocks of varied kinds, which have in fact now been "made to order" in a number of instances, in order to procure needed tools for other genetic work.

AN ANALYSIS OF THE EFFECTS OF THE DIFFERENT RAYS OF RADIUM IN PRODUCING LETHAL MUTATIONS IN DROSOPHILA[1]

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EXPOSURE of fruit flies to radium rays produces both lethal and visible mutations. With heavy treatment the lethals are so numerous as to lend themselves to exact quantitative analysis.

Hanson and Heys[2] showed that when flies were treated for six hours with 140 milligrams of radium the percentage of lethal mutations was 8.2 per cent. And, further, that when the alpha and beta rays were completely absorbed by a lead filter the gamma rays alone produced 2.8 per cent. of lethals, although in this latter case it is highly probable, in the light of work reported below, that it was a secondary radiation of beta particles produced by the passage of the gamma rays through matter that was the effective agent in causing mutations.

In unpublished work on visible mutations due to radium rays it has been found that the gene mutations are, in general, in the same direction and of the same nature as those occurring spontaneously in the fruit fly and also parallel exactly the results obtained by Muller[3] with X-rays.

Darwin—and most writers on evolution since—have emphasized the importance of discovering the cause or

[1] Address at the meeting of the Genetics Section of the American Society of Zoologists, New York City, December 28, 1928.

[2] Frank Blair Hanson and Florence Heys, "The Effects of Radium in Producing Lethal Mutations in *Drosophila melanogaster*," *Science* 68: 115–116 (1928).

[3] H. J. Muller, "Artificial Transmutation of the Gene," *Science* 66: 84–87, 1927; "The Production of Mutations by X-rays," *Proc. Nat. Acad. Sci.*, 14: 714–726, 1928; "The Problem of Genic Modification," *Verhandlungen des V. Internationalen Kongresses für Vererbungswissenschaft*, 234–260. Berlin. 1928.

causes of variation. It has become a trite statement in evolutionary literature that the central problem of evolution lies in the cause of variability and that we know nothing about agents causing variability. And this situation remained practically unchanged from 1859, the date of the publication of the "Origin of Species," until the summer of 1927, when Muller[3] announced that X-rays were effective agents in producing gene mutations in Drosophila. This marked a milestone in biological progress. Stadler,[4] Weinstein[5] and Hanson[6] soon followed with confirmations of Muller's work; Stadler,[4] and Hanson and Heys[2] extended it to include radium irradiation.

On the other hand, investigations by many workers over a long period of years in which genes were exposed to numerous chemicals, high and low temperatures and ultra-violet light had given only negative or inconclusive results. The importance of the work with X-rays and radium lies in the fact that of all conceivable agents which might possibly cause gene mutations the field has been narrowed down to two physical agents, X-rays and radium, which under experimental conditions are highly effective in this respect. This is suggestive, at least, as Muller has pointed out, that variability in organisms *in nature* may be due to natural X-rays and other forms of radiation. It is not inconceivable that the relatively infrequent occurrence of natural mutations as compared with the greatly accelerated rate under experimental irradiation is but the reflection of the difference in amount of rays present in the two situations.

As indicated above, narrowing the field of possible highly effective mutative agencies to X-rays and radium

[4] L. J. Stadler, "Mutations in Barley Induced by X-rays and Radium," *Science* 68: 186–187, 1928.

[5] Alexander Weinstein, "The Production of Mutations and Rearrangements of Genes by X-rays," *Science* 67: 376–377, 1928.

[6] Frank Blair Hanson, "The Effects of X-rays on Productivity and the Sex Ratio in *Drosophila melanogaster*," AM. NAT., 62: 352–362, 1928; "The Effect of X-rays in Producing Return Gene Mutations," *Science* 67: 562–563, 1928.

is of primary importance, for it focuses endeavor where results of significance seem assured. However, a still further analysis into the ultimate cause of variation may be possible. The product of an X-ray tube is not a single homogeneous kind of ray, but a complex of many things. Again, as is well known, radium gives off at least three different kinds of rays, namely, the so-called alpha, beta and gamma rays. Each of these may include rays of differing velocity, wave-length and penetrating power. The questions then arise: does it take all the elements in X-rays or all three of the radium rays acting together to induce the gene to mutate; or is some particular combination of two or more of these different types of rays necessary; or, finally, is there just some *one* unit common to both X-rays and radium to which all the mutations are due? This paper is a presentation of evidence indicating that the latter is probably the case.

Thus far in the work with radium the alpha rays have been entirely excluded. The silver needles of radium used emit only beta and gamma rays. So it can be said at the outset that alpha rays are not necessary for the production of mutations. This does not imply, of course, that alpha rays may not also induce gene changes, but merely that no flies have been subjected to their action. Whether or not alpha rays acting *alone* are effective is one of the problems of the immediate future. The technical difficulty of getting a pure source of alpha radiation has been very great, but a start has been made toward its solution. At the present time, then, we only know that, whatever the effect of alpha particles may ultimately prove to be, the production of gene mutations is possible without their aid.

Breeding Process

Wild type males were exposed to 150 milligrams of radium[7] for nine hours. The shell vials, two and one

[7] The radium used in these experiments was loaned by the Barnard Free Skin and Cancer Hospital of St. Louis. Our appreciation is hereby expressed for this courtesy.

half by one inches, in which the flies were confined, contained one inch of cooked banana-agar food, and were covered at the open ends with two layers of gauze. The radium needles were attached with adhesive tape to lead filters of varying thicknesses which were interposed between the flies and the radium. There were nine such treatment vials, each containing approximately 175 males. One vial of flies received no treatment, being held as controls. Each of the vials had a lead filter of one of the following thicknesses (in inches): .000, .005, .010, .019, .078, .156 and .312, over its open end.[8]

Immediately following treatment with radium these males were mated, in pairs, to females having the genes for scute (sc), vermilion (v), forked (f), and bobbed (bb) in one X-chromosome and a gene (C) to prevent crossing over, a lethal gene (l), and the bar gene (B) in the other X-chromosome. The F_1 females were of two classes, bar and not-bar. The bar-eyed females were mated to their brothers, one pair to a tube, to produce the F_2 generation. Due to the presence of the lethal gene in half of the sons in this generation they never hatch. If a new lethal mutation arose as the result of the radium treatment the other half of the sons would inherit it and not appear. Hence no males would hatch in that particular tube. To determine the number of lethal mutations occurring as a result of the exposure to radium it was only necessary to count the number of tubes in which there was 100 per cent. females. Figure 1 is a graphic representation of the breeding process just described.

BREEDING RESULTS

The first four columns in Table I give the breeding results of the experiment; the last three columns, the ionization data which will be described below.

[8] The lead filters were provided through the courtesy of the Hoyt Metal Company of St. Louis, and their accuracy checked by the Engineering Department of Washington University.

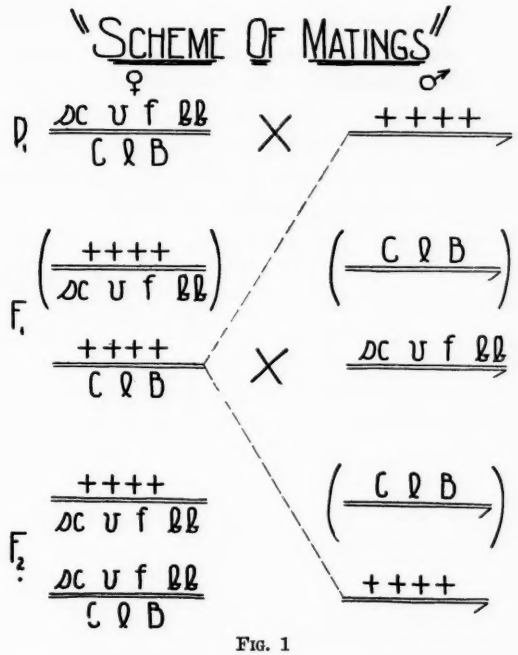

FIG. 1

The first column in the table gives the thicknesses (in inches) of the lead screens. Since commercial houses dealing in lead do not use the metrical system their des-

TABLE I

"BREEDING DATA"				"IONIZATION DATA"		
THICKNESS FILTER (INCHES)	NO F_2 TUBES	NO. LETHAL MUTATIONS	PERCENT. MUTATIONS	FALL GOLD LEAF (SEC)	IONIZATION PROPORT. TO	PERCENT. MUTATIONS
000	DR I-123 / DR II-28	16 / 4	13.6	9.6	.104	13.7
005	1045	99	9.4	13.8	.072	9.4
010	600	46	7.6	17.3	.058	7.6
019	539	34	6.0	21.8	.046	6.0
039	514	24	4.6	28.5	.035	4.6
078	697	24	3.4	38.0	.026	3.4
156	1370	35	2.5	59.3	.017	2.2
312	862	9	1.0	96.0	.010	1.3
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ignation of the thickness of the lead plates has been retained. Reading down the first column it will be noticed that each filter doubles in thickness over the preceding one. The first lead filter (.005) is approximately one eighth of a millimeter thick. In column 2 the number of fertile F_2 pairs of parents is given for each respective thickness of lead screen, while columns 3 and 4 give the actual number of lethal mutations and the percentage.

When no filter (.000) intervenes between the radium and the flies the percentage of mutations is 13.6. At the other end using .312 inches of lead there is only 1 per cent. of lethals. For filters intermediate between these extremes there is a regularly graded series of mutations (see Table I). In other words, the per cent. of lethal mutations decreases with increasing thicknesses of lead.

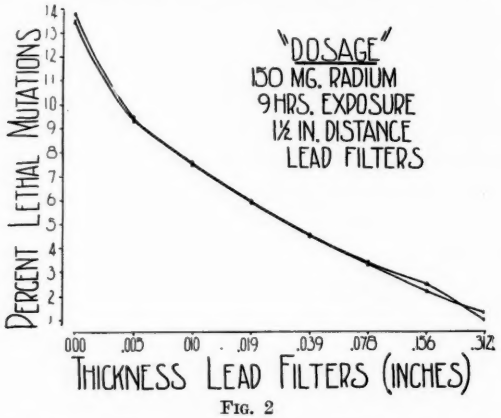

FIG. 2

The resulting curve is shown in Figure 2. The 423 control tubes gave no lethal mutations.

MEASUREMENT OF IONIZATION

During the course of the mutation experiments a parallel series of experiments was conducted using the tools of the physicist to measure the amount of ionization in air produced by radium rays passed through the same series of lead filters used in treating the flies with radium.

If, as is believed, these mutations are the result of radium rays being absorbed by the organism, then it is only to be expected that the number of mutations be directly proportional to the number of rays to which the organisms are exposed. This becomes less, the thicker the protecting lead filter becomes.

But how is the absorption through these filters of varying thickness to be measured? The physicist uses this method of attacking the problem: when beta rays are absorbed in a gas, such as air, the molecules of the gas are "ionized," that is, a small particle charged with negative electricity is torn from the molecule, leaving the rest of the molecule charged with positive electricity. These charged particles, both negative and positive, are

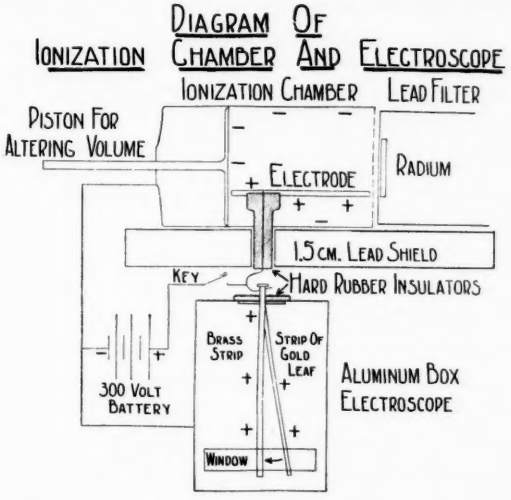

FIG. 3

called "ions," and the rate of production of these ions is proportional to the intensity of the radiation. Then if we measure the amount of ions produced, we measure the absorption taking place.

Ionization is measured with an ionization chamber and electroscope, as shown in Figure 3. In this chamber, ionization due to the radium takes place as just described.

The chamber is fitted with a movable piston arranged so that the effective volume of the air in the chamber can be altered at will. In the chamber is a wire electrode supported on a hard-rubber insulator, through which a fine wire leads to the electroscope. Between the ionization chamber and electroscope is a 1.5-cm lead shield to prevent rays from the radium penetrating into the electroscope.

The electroscope is a device which measures the ionization taking place in the chamber above. It consists simply of a small aluminum box inside of which is a narrow brass strip to which is attached at the top a narrow strip of fine gold foil. These strips are insulated from the box by means of a hard-rubber cap, but are connected by a fine wire to the electrode above.

If now the ionization chamber and aluminum case are connected to the negative pole of a 300-volt battery, and the electrode and strips are touched by a wire from the positive pole of the battery, the gold leaf will stand out from the brass strip, due to the fact that the two strips, being both positively charged, repel one another.

When a needle of radium is placed near the open mouth of the ionization chamber the rays enter and ionize the air. The negative ions are attracted to the positive electrode (due to the fact that negative and positive electricity attract one another). But upon touching it, they partly neutralize the positive charge of the electrode. Since the electrode is connected to the electroscope, the charge on the brass and gold strips is also lessened, and the leaf falls back toward the brass strip. The greater the ionization, the faster the leaf falls. The rate of fall is determined by observing the gold leaf through a short-focus telescope pointed toward a small window cut in the aluminum box. There is a 5-cm scale in the eyepiece of the telescope, and a stop-watch is used to time the fall of the gold leaf over this 5-cm scale. A definite number of ions will be produced per second by the radiation, but as increasingly thick filters are intro-

duced between the radium and ionization chamber fewer rays get into the chamber and there are fewer ions produced. Therefore the charge on the electroscope is neutralized more slowly, and a greater time is required for the leaf to fall five centimeters. Ionization is inversely proportional to the time required by the gold leaf to move across the 5-cm scale. That is, we get the ionization by dividing into one the number of seconds required for the fall. The values thus obtained, however, are only relative values. The ionization curve can be fitted arbitrarily to the mutation curve at one point, say the point where the filter used was .019 inches thick. Here the mutation rate was 6 per cent., while the value of ionization is proportional to .046. If we let .046 represent 6 per cent., the percentage of mutations represented by the other values can easily be calculated. Thus .058 represents $.058/.046 \times 6 = 7.6$, this value corresponding exactly with the per cent. of mutations found using a filter .010 inches thick. The other values in the last column of Table I have been computed similarly.

When the ionization experiments were performed the mutation percentages already had been determined for the following series of lead filters: .005, .010, .019, .039 and .078. It will be noted from Table I and Figure 2 that these percentages are precisely the same as the ionization values obtained for these filters. Prior to determining the mutation values for .000, .156 and .312 inches of lead, the ionization data were secured for these three points and on the basis of those data it was predicted what the number of mutations would be for those points on the curve. Hence, using no filter at all (.000) the ionization value was 13.7; at .156 it was 3.4, and for .312 it was 1.3. And the prediction was made that the mutation percentage for these points on the curve would be close to or exactly the same as the ionization values. When the breeding results were in it was found that there was a difference of one tenth of 1 per cent. between the two values at point .000 and a difference of three tenths

of 1 per cent. at the points .156 and .312. Of the eight points on the curve, then, the correspondence between the mutations and ionization values is perfect for five points and of the other three points the maximum departure is only three tenths of 1 per cent. For all practical purposes the two curves are exact fits, yet one is a biological curve based on breeding results, the other a physical curve based on the ionization of air by radium rays.

By these methods it becomes quite possible to predict with great accuracy upon the basis of our knowledge of the absorption power of lead as measured in an electroscope the number of lethal mutations that may be expected in fruit flies. Contrariwise it is equally feasible to use mutation data of the kind employed here as a rather exact means of measuring or predicting the ionization rate of radium rays in air.

DISCUSSION

As shown above, radium radiation produces lethal mutations in fruit flies and the per cent. of mutations is dependent upon the thickness of the filter. This brings us to the problem of the essential agent in radium and X-rays responsible for the results. It will be recalled that the alpha particles are already ruled out, for they were all absorbed by the silver needles containing the radium. This leaves the beta and gamma rays for consideration.

Gamma rays, according to Rutherford,[9] are extraordinarily penetrating rays, considerably more penetrating, in fact, than the X-rays produced in a "hard" vacuum tube. They are, nevertheless, analogous in their properties to the X-rays. They have the further characteristic, in contrast with alpha and beta rays, of being nondeviable in a magnetic field. Again, the ionization or electric effect directly due to the gamma rays is very feeble in comparison with those of the beta rays. In fact, experiments by English radiologists, as quoted by

[9] E. Rutherford, "Radioactive Substances and Their Radiations." Cambridge, vii + 699 pp. 1913.

Rutherford,[9] indicate that the ionization in an enclosure is due to the excited beta rays and not to the gamma rays themselves. Bragg[10] has investigated the question for X-rays, and concludes that on the whole the evidence indicates that ionization is due to beta radiation and not to the X-rays themselves. This is important in view of the fact that both X-rays and radium produce gene mutations.

The last characteristic of the gamma rays is that when these very penetrating, non-deviable rays pass through any kind of matter, such as the lead filter or the body of the fly, there is set up a secondary beta radiation. It is also true that the passage of X-rays through matter is always accompanied by the appearance of high-speed electrons or beta rays. Since beta rays ionize a gas, while gamma rays and X-rays do not to any extent do so, the ionization results described above are mainly, perhaps wholly, due to the primary and secondary beta radiation.

In the series of lead filters thinner than .078 inches, primary beta rays are present in the ionization chamber and in the fly culture tubes while under treatment, whereas, above and including .078, all primary beta radiation is held back by the filters. But the air is ionized, nevertheless, by the secondary beta rays excited by the gamma rays which readily pass through all filters of the thicknesses used here. And it seems to make no difference either in the number of mutations or amount of ionization whether the beta radiation is primary, *i.e.*, direct from the radium itself when thin filters are used, or secondary, from gamma rays, as when the filters are .078 inches, or greater, in thickness.

Since only the beta rays ionize the gas in the ionization chamber and the ionization curve fits the mutation curve so perfectly, it seems safe to conclude that the gamma rays are ruled out as directly causing mutations. This

[10] W. H. Bragg, "Effects of X-rays in Ionization" (not exact title), *Proc. Roy. Soc.* A. 85: 349–365, 1910.

would then be true for X-rays also, as they, like gamma rays, do not ionize a gas except as they are the source of a secondary beta radiation. We have earlier eliminated the alpha rays. The beta rays are alone left for consideration.

Apparently all the evidence points to the conclusion that the essential and direct agent in the production of mutations is the negatively charged, high-speed beta particle. These beta particles are given off by radium and also excited in matter traversed by either gamma rays or X-rays. This explains the success and identical results obtained by Muller and others with X-rays and Stadler, Hanson and Heys with radium. Probably also the almost total lack of success with agents other than these two.

If, as suggested, the beta particle is the effective agent involved in gene changes, then the cause of variability in fruit flies, and possibly in all organisms, has been tracked down to the ultimate entity known to physics—the electron.

It is interesting to note that the fundamental problem of biological variability necessarily waited for its solution upon the most recent developments in physics, again illustrating the interdependence of the arbitrarily delimited fields of science. Genetics has joined general physiology in furnishing a common meeting ground for biology and physics.

CONCLUSION

Drosophila flies exposed to 150 milligrams of radium for a period of nine hours show a 13.6 percentage of lethal mutations. Interposing a series of lead filters between the radium and the flies gives a decreasing mutation rate dependent upon the increasing thickness of the filters, and a mutation curve can be constructed showing this relationship.

When radium rays are passed through the same series of filters into an ionization chamber, the air is ionized

and the connecting electroscope is discharged. The ionization value for each filter can be computed and these data used to plot an ionization curve, which if made arbitrarily to fit the mutation curve at one point also fits it with a remarkable exactitude at the other seven points.

Since all alpha particles were held back by the silver in the radium needles and the gamma rays do not ionize a gas, except indirectly through excited beta radiation, alpha and gamma rays are excluded.

Ionization is due to the beta radiation, and since the ionization curve can be superimposed upon the mutation curve the evidence is all but complete that the beta particle is the effective agent in gene change. These results apply to X-rays as well as radium.

Another method of determining this has just now been made available. Beta rays are identical with the negatively charged particles constituting the cathode or Lenard rays. As this is being written a Lenard ray tube has been received for use in a series of experiments similar to those reported here with radium.

EVIDENCE THAT "UNISEXUAL" PROGENIES IN SCIARA ARE DUE TO SELECTIVE ELIMINATION OF GAMETES (SPERMS)[1]

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IT was shown in earlier papers (Moses and Metz, 28, Metz and Moses, 28) that in the "unisexual" progenies found in Sciara the sex of the progeny is determined by the genetic constitution of the mother; and that the inheritance of the differentiator between "male progenies" and "female progenies" appears to follow a simple Mendelian scheme. Subsequently it was shown (Metz, 29) that the inheritance of a sex linked character in *S. coprophila* (which gives "unisexual" progenies) indicates the presence here of a pair of sex chromosomes of the usual type in which the female is XX and the male XY.

The latter finding would in turn indicate that the X and Y chromosomes are sex determining and also, since a male gives both sons and daughters, that the two corresponding types of sperms (X-bearing and Y-bearing) are regularly produced by each male. It has also been shown that no parthenogenesis occurs.

Considering the above lines of evidence together, it is obvious that if the male produces the two types of sperms and yet his offspring by any one female are of only one sex, there is a selective elimination of the other sex to be accounted for. This could be due to a selective elimination of zygotes or of gametes (sperms). The simpler hypothesis is that of elimination (differential mortality) of zygotes. On such a basis it would be assumed that the eggs of a female are fertilized at random by both types

[1] This investigation has been aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

of sperms and that the males or the females, as the case may be, fail to develop. Since female-producing females and male-producing females are genetically different, it would be assumed that the eggs of the two also differ, and in such a way as to make them capable of complete development only in connection with the appropriate type of sperm—*i.e.,* in female progenies the male embryos would die and in male progenies the female embryos would die.

If differential mortality of this sort occurs it must, obviously, be at some period between the fertilization of the egg and the emergence of the adult fly. Therefore, its presence should be detected by counting the eggs soon after fertilization and comparing with the total number of flies developing from them. The number of flies should not exceed 50 per cent. of the number of eggs.

The present paper deals primarily with experiments designed to test this hypothesis. These experiments deal separately with two periods, that preceding the time of egg laying and that following this time. If fertilization occurs at the time of egg laying, as the evidence seems to indicate, the later period is the only one of importance in the present connection. But since the exact time of fertilization has not yet been determined, both periods have been included in the observations. For the sake of completeness a few additional notes concerning the history of the eggs have also been included.

The species used is *Sciara coprophila* Lint.

History of the Eggs After They are Laid

To test the mortality rate after the eggs are laid seven experiments were made in various ways, as outlined below. In some cases the counts were checked by two or three persons independently. For assistance in this the writer is indebted to Dr. Helen Monosmith, Mr. Adrian Ter Louw and Miss Louise Schmuck.

The data from these experiments are summarized in the accompanying table.

Experiment 1 served partly as a preliminary test of methods and is incomplete in certain respects. Here the fertilized females were put in vials on plain agar (one female to each vial) and permitted to lay their eggs, after which they were removed and the eggs counted under the Greenough binocular. The eggs in each case were counted three times and the counts averaged. The counts were consistently in close agreement, varying by not more than four or five. These cultures were then fed and cared for in the usual manner and the flies counted when they hatched. In this experiment the sexes were not recorded in detail, but the sex of the progeny as a whole was indicated in most cases (last column). The percentage of flies hatched as compared with eggs counted is given in the next to the last column.

It will be seen at once that in most of these counts the number of flies is much more than half the number of eggs. In only three of the fourteen counts was the number less than 60 per cent., and in seven it was over 70 per cent. In number 14 it exceeded the number of eggs counted, showing that some of the eggs were overlooked.

Experiment 2 was essentially a repetition of experiment 1, but was made with special attention to the possibility of overlooking eggs in making the counts. The eggs were counted by Dr. Monosmith and the flies by the writer. In this and subsequent experiments the sex of each fly was recorded.

Experiment 3 differs from the preceding only in that the eggs were counted by both Dr. Monosmith and the writer independently. The counts were in close agreement and where different the larger one was used in the records.

In both these experiments the results are substantially the same as in the first. The only question would seem to be the possibility that many eggs were overlooked in making the counts. There appeared to be no chance for this to occur, with the methods used, but in order to make the point certain, more elaborate tests were made.

In *experiment 4* the cultures were made with agar colored black for contrast and then the peripheral part of the exposed surface of the agar was covered with a ring of melted paraffin, leaving only a small central area exposed for egg laying. After the female was removed the ring of paraffin was lifted out and the eggs, all clearly exposed near the center, were counted. The counts were made by the writer and checked by Miss Schmuck. Here again some of the cultures showed far more than half of the eggs completing development to mature flies.

As a final test, *experiment 5* was carried out. Here the eggs were transferred to the cultures by means of a needle so as to make absolutely sure of the counts. The numbers are necessarily small, for this reason, but they definitely confirm the preceding observations. The eggs all came from two original lots, both of which happened to be from female-producers, which accounts for all the flies being females.

To make the critical data more extensive, duplicates of experiments 4 and 5 were carried out by Dr. Monosmith, with similar results, as shown in *experiments 6* and *7*.

The above experiments are believed to show without question that the "unisexual" progenies in *Sciara* are not unisexual because of any differential mortality operating after the fertilized eggs are laid. If there is any such mortality, therefore, it must take place between the time the egg is inseminated and the time it is laid. This possibility is considered below. Owing to the importance of the point at issue here, the observations are given in considerable detail.

History of the Eggs Before They are Laid

Since we are concerned here only with the question of egg mortality after fertilization, two methods of investigation are available. We may ascertain the exact time at which the eggs are fertilized and then follow their history from this point, or we may ignore the time of fertili-

TABLE I

COUNTS OF EGGS LAID AND MATURE FLIES OBTAINED IN *Sciara coprophila*. EACH COUNT IS FROM ONE MOTHER

Experiment 1. (Ter Louw)

No.	Eggs	Flies	Per cent.	Sex
1	118	42	35.6	♀
2	125	79	63.2	♂
3	90	85	94.5	♀
4	60	53	88.3	♀
5	80	58	72.5	♂
6	146	140	95.7	♂
7	141	127	90.0	
8	115	69	60.0	♂
9	147	99	67.3	
10	87	64	73.5	♂
11	150	74	49.3	♂
12	11	7	63.6	♀
13	117	66	56.5	
14	75	78	104.0	♂

Experiment 2. (Monosmith)

No.	Eggs	Flies		Per cent.
		♀	♂	
1	175	0	83	47.4
2	175 ± 5	0	150	85.7
3	120	0	29	24.2
4	153	88	0	57.5
5	187	0	75	40.1
6	99	0	88	88.9
7	132	0	83	62.9
8	15	0	4	26.7
9	150	0	81	54.0
10	142	142	0	100.0
11	189	137	0	72.5
12	25	12	0	48.0

Experiment 3. (Monosmith & Metz)

No.	Eggs	Flies		Per cent.
		♀	♂	
1	31	15	0	48.4
2	52	0	23	44.2
3	60 ± 5	52	1	88.3
4	73	64	1	89.0
5	173	90	0	52.0
6	118	0	42	35.6

TABLE I—(*Continued*)

COUNTS OF EGGS LAID AND MATURE FLIES OBTAINED IN *Sciara coprophila*. EACH COUNT IS FROM ONE MOTHER

Experiment 4. (Metz & Schmuck)

No.	Eggs	Flies ♀	Flies ♂	Per cent.
1	149	97	0	65.1
2	113	1	81	72.6
3	113	98	1	87.6
4	184	86	9	51.6

Experiment 5. (Metz)

Eggs Transferred with Needle

No.	Eggs	Flies ♀	Flies ♂	Per cent.
1	20	16	0	80.0
2	18	14	0	77.8
3	21	16	0	76.2
4	38	26	0	68.4
5	46	16	0	34.8
6	29	26	0	89.7

Experiment 6. (Monosmith)

No.	Eggs	Flies ♀	Flies ♂	Per cent.
1	221	57	0	25.8
2	203	2	175	87.2
3	59	41	0	69.5
4	153	0	90+	58.8
5	213	0	102	47.9
6	92	0	23	25.0
7	180	184	0	102.2
8	114	0	50	43.9
9	252	140	0	55.6

Experiment 7. (Monosmith)

Eggs Transferred with Needle

No.	Eggs	Flies ♀	Flies ♂	Per cent.
1	118	61	0	51.7
2	50	1	0	2.0
3	116	99	0	85.3

zation of the eggs and follow their history from the time the female mates. Both of these methods have been used to some extent, but since the former has led to a detailed microscopic study which can not be completed soon, attention will here be given mainly to the latter.

In this connection it is necessary to note certain general characteristics concerned with reproduction in *Sciara.* Most important, perhaps, is the fact that in the species used, as well as all others thus far studied by the writer, the eggs of a given female develop synchronously, mature at practically the same time and are usually all laid within a relatively short period (see below). The eggs are well developed when the female emerges from the pupa, but are apparently not fully grown and not mature at this time. Copulation may occur at any time after the female emerges.

While not final, present evidence points toward the conclusion that in *Sciara,* as has been found by Nonidez (20) to be the case in *Drosophila,* the eggs are inseminated one by one as they pass from the oviduct immediately before they are laid. The gross morphology of the reproductive system appears to be much the same in the two groups, and the writer has found by repeated observation that in *Sciara,* as in *Drosophila,* the eggs retain their original positions in the ovaries until they are about to be laid. Each egg lies in a separate follicle. If fertilization occurs more than a few minutes before laying, therefore, it must occur while the egg is still in this position in the ovary. No micropyle has yet been identified, but it may be noted that if the micropyle is at the distal end of the egg (farthest from the oviduct) as in *Drosophila,* it seems unlikely that the sperm could reach it until the egg was released from the follicle immediately before being laid.

Two other lines of evidence also point to the conclusion that fertilization accompanies egg laying. One is the fact that eggs fixed within three or four hours after laying have shown only a few cleavage divisions, and that

sectioned ovarian eggs, from a (presumably) fertilized female which had been with males for three days and was about to lay, showed no signs of development. The other is the fact that visible changes indicate that the eggs are not mature until approximately the time they are laid, which is supported by the fact that if mating is prevented until the eggs are mature (two or three days) fertile eggs will be laid just as soon as if mating occurred within a few hours after emergence.

Whatever the exact time of fertilization of the egg, it is clear from experimental evidence that it does not necessarily occur more than twenty-four to forty-eight hours (perhaps less) preceding laying. If a 50 per cent. mortality occurs during this period it must be assumed that the affected eggs either completely degenerate and disappear, or that they remain in the female, since it has been shown that they are not laid. It seems improbable that they could degenerate completely in this length of time, but if they do it should be easy to detect the process by merely examining the eggs of females at successive stages after copulation.

This has been done in two ways, first by random examination of females of different ages from mass cultures where plenty of males were present and second by examination of females of known ages exposed to males at definite times. The evidence presented below is supported by numerous incidental observations made in connection with earlier experiments but not included here.

The detailed observations dealing with these points are outlined below. They were made by the writer.

Experiment 8. In this experiment fifteen virgin female-producing females were used. They came from two mothers and were approximately the same age—having been taken in the morning from cultures from which all adult flies had been removed the evening before. These females were divided into three lots of five each (*A, B, C*) to which males were added as follows:

A Ten males added at once – 10 A. M. first day
B Ten males added after 24 hours – 10 A. M. second day
C Ten males added after 48 hours – 10 A. M. third day

No eggs had been laid by any females up to the time males were added to *C* (10 A. M. third day). At this time one female was removed from each lot and dissected. These were recorded as A–1, B–1 and C–1. The eggs appeared to be in the same condition in all three. They were in their follicles in the ovaries, apparently fully mature and ready to be laid, with no signs of degeneration.

At 4 P. M. on the third day, all males were removed and discarded and the females isolated singly in separate cultures, A–2, A–3, etc.; B–2, B–3, etc.; C–2, C–3, etc. Thus females in lot *A* were exposed to males from the time they were a few hours old until they were about sixty hours old. Those in lot *B* were exposed for thirty hours beginning when they were about twenty-four to thirty hours old. And those in lot *C* were exposed for six hours beginning when they were approximately fifty hours old.

At about 10 A. M. of the fourth day female A–2 was dissected. Her eggs were apparently fully mature, *in situ* in the ovaries, just as in those dissected the preceding day. There were no signs of degenerating eggs. At this time female B–2 was dead and C–2 lost. The remaining nine females were transferred to new cultures—A–3(2), A–4(2), etc.

At about 10 A. M. on the fifth day the females were again transferred to new cultures—A–3(3), A–4(3), etc. (final transfer). Eggs were visible at this time in cultures B–4 and C–4(2).

All cultures were then left undisturbed until the morning of the seventh day, when the females (all dead, except one) were removed and dissected. Examination of the ovaries and oviducts revealed the conditions summarized below:

A–3(3) dead; ovaries, etc., empty; no sign of degenerating eggs.
A–4(3) dead; many fully developed eggs in ovaries; none degenerate.
A–5(3) alive; ovaries, etc., empty; no sign of degenerating eggs.
B–3(3) dead; many fully developed eggs in ovaries; none degenerate.
B–4(3) dead; fifteen mature eggs in ovaries; no sign of degenerating eggs.

B–5(3) female lost.
C–3(3) dead; nine mature eggs in ovaries; no sign of degenerating eggs.
C–4(3) dead; ovaries, etc., empty; no sign of degenerating eggs.
C–5(3) dead; many mature eggs in ovaries; no sign of degenerating eggs.

All cultures were kept until the fifteenth day, at which time the larvae were well developed, making it easy to see which cultures had received fertile eggs. The six females eliminated (by dissection or otherwise) on or before the fourth day gave no offspring. Of the remaining nine, all but one (A–3) gave offspring. Larvae were found in cultures A–4(2), A–5(3), B–3(2), B–3(3), B–4, B–5(3), C–3(3), C–4(2), C–5(2) and C–5(3), but not in any of the others.

From these observations it appears that of the three females exposed to males first and longest, A–3 laid only infertile eggs, A–4 laid fertile eggs when it was between three and four days old and A–5 laid fertile eggs when it was four or more days old. In the *B* series B–4 laid fertile eggs at an age of approximately two and a half to three days and the other two at an age of four days or more. In the *C* series, exposed to males for only six hours and at an age of approximately two and a half days, two females laid fertile eggs at the age of between three and four days (also later in case of C–5) and two at four days or more. The similarity of the results in all three series indicates that the eggs are probably not ready for fertilization until the female is at least two days old.

In these experiments the flies were kept at room temperature, with low temperatures at night. At higher temperatures the time periods would be shortened.

Experiment 9. For this experiment fifteen females from "unisexual" stock (M 674) were selected at random, except that old females which had obviously laid their eggs were avoided. These females had been with a few males and about forty additional males were added at once.

After about forty-eight hours, many eggs were visible in the culture, so nine of the (apparently) oldest females were removed and dissected, with the following results:

(1) 34 mature eggs in ovaries; no sign of degenerating eggs.
(2) 150 ± mature eggs in ovaries; no sign of degenerating eggs.
(3) 75 ± mature eggs in ovaries; no sign of degenerating eggs.
(4) 100 ± mature eggs in ovaries; no sign of degenerating eggs.
(5) 4 mature eggs in ovaries; no sign of degenerating eggs.
(6) 32 mature eggs in ovaries; no sign of degenerating eggs.
(7) 100 ± mature eggs in ovaries; no sign of degenerating eggs.
(8) Ovaries full of mature eggs; no sign of degenerating eggs.
(9) 11 large and 31 considerably smaller, but not visibly degenerating, eggs in ovaries. Female dead.

On the next day many more eggs were visible in the culture and three of the remaining six females were dead. All six were dissected and examined, with the results indicated below.

(10) Dead; ovaries, etc., entirely empty.
(11) Dead; 27 mature eggs in ovaries, none degenerate.
(12) Dead; ovaries, etc., entirely empty.
(13) Alive; ovaries full of mature eggs; none degenerate.
(14) Alive; about 100 mature eggs in ovaries; none degenerate.
(15) Alive; about 150 mature eggs in ovaries; none degenerate.

Experiment 10. For this experiment females were taken from a stock bottle in which nine females and sixteen males were present (M 674 stock). The females were isolated singly in separate cultures and left for three days. At the end of that period the females were removed and dissected and the cultures examined for eggs. Where eggs were present in the cultures they were examined under the microscope. The observations are summarized below.

(1) ♀ dead; ovaries and ducts entirely empty.
Many eggs in culture; all good; no sign of degeneration.
(2) ♀ dead; 6 mature eggs in ovaries; otherwise empty.
Eggs in culture just as in preceding.
(3 and 4) Conditions in females and in cultures just as in preceding, except one female has 11 and other 12 eggs in ovaries.
(5) ♀ alive; 1 ovary empty; other full of mature eggs.
No eggs visible in culture. Presumably had laid eggs before isolation.

(6) ♀ dead; ovaries full of mature eggs; no sign of degeneration.
No eggs visible in culture.
(7) ♀ dead; ovaries, etc., entirely empty save for 1 mature egg.
Many eggs in culture; all normal in appearance.
(8) Just as in (7) except 19 eggs instead of 1 in ovaries.
(9) ♀ alive; ovaries entirely empty; body full of fat.
No eggs visible in culture.

From cultures (5), (6) and (9) no flies were obtained. The others gave the following counts: (1), (3) and (8) approximately 50 ♂♂ each, no ♀♀; (2) 3 ♀♀, no ♂♂; (4) 10 ♂♂, no ♀♀; (7) approximately 40 ♀♀, no ♂♂.

Experiment 11. This experiment is essentially like the preceding except that the flies are from another stock (3086). Three females were isolated singly from a stock bottle in which many more males than females were present. After two days one female had laid her eggs and died. Her ovaries and ducts were entirely empty and the eggs in the culture all apparently normal. On the next day eggs were visible in a second culture. These also appeared entirely normal. Dissection of the female (alive until killed for dissection) showed the ovaries and ducts entirely empty. On the following day the third female was killed and dissected, although no eggs were visible in the culture. Her ovaries and ducts were empty save for 27 mature eggs. Presumably she had laid her eggs before isolation. Counts from these three cultures were recorded as follows: (1) $50 \pm$ ♀♀; (2) 0; (3) $100 \pm$ ♂♂. Presumably the records for (2) and (3) were interchanged by mistake, but the error was not noticed until after the cultures were discarded.

In considering the above observations it should be noted that when the eggs reach full growth and are presumably mature they slip readily from their follicles if the ovary is manipulated with the dissecting needle. This behavior, as well as size and appearance, has been used as a criterion of maturity. It should also be noted that in many cases a few oocytes in the ovary fail to develop. These remain very small, as compared with the eggs, are translucent in appearance, have large nuclei

clearly visible at all stages, remain firmly fixed in the ovary and present the same appearance in old flies that have laid their eggs as in newly emerged ones. Since they can not be confused with the eggs and since they are frequently almost or entirely lacking, they are ignored in the above records.

Owing to the elasticity of the follicular walls or membranes, the ovary contracts as the eggs are laid until, after all are laid, it is only a small fraction of its previous size and consists of practically nothing but an almost transparent membrane. For this reason its contents, if any, are clearly visible and the presence of degenerating eggs would be readily detected at any stage.

The material used in the above experiments involves three different lines, all of the "unisexual" sort typical of this species. In experiment 8 all the females are female-producers. They are from an experimental line in which female-producers may be distinguished from male-producers by means of a mutant character, the gene for which appears to be completely linked to the differentiator determining the "sex of the progeny as a whole" (unpublished data).

In these experiments females of various ages have been dissected at sufficiently short intervals between the time of mating and that of egg laying to make it seem certain that any considerable mortality and degeneration of eggs during this period would be detected. Since no evidence of such degeneration has been found, it is concluded that no selective elimination of eggs occurs here which could account for the regular production of "unisexual" progenies. Likewise, it has been found that no regular retention of eggs by the female occurs and hence it is concluded that selective elimination, of the kind under consideration, is not effected in this manner.

In addition to the above, a comparison has also been made between the maximum number of eggs produced by females and the maximum number of offspring obtained. Such a comparison is not critical, because it is impossible

to use the same fly for both observations, since the egg counts are obtained by dissecting newly hatched females. Nevertheless, if a 50 per cent. mortality occurred, there should be a wide discrepancy between the two maxima, and this has not been true of the counts. The larger egg counts range between three hundred and four hundred and the larger progeny counts range around three hundred.

Discussion and Summary

It is believed that the above observations warrant the conclusion that the regular production of "unisexual" progenies in *Sciara coprophila* is not due to a differential mortality of zygotes. The evidence involves three separate features: (1) the possibility of such mortality occurring after the eggs are laid; (2) the possibility of its being brought about by a selective retention of fertilized eggs by the female, and (3) the possibility of its being due to degeneration of fertilized eggs within the body of the female. Evidence on the first two features is clear cut. It shows that no such mortality as would be required occurs after the eggs are laid, and likewise that eggs are not regularly retained by the female. That concerning the last feature is negative in nature, being based on the lack of any indications of degeneration, but it appears, nevertheless, to be equally conclusive.

If our original premises are correct the only alternative seems to be the assumption that a selective elimination or inactivation of sperms occurs, such that in female-producing females the Y-bearing sperms do not function and in male-producing females the X-bearing sperms do not function (save for occasional exceptions in each case). There is no evidence at present to indicate whether this elimination or inactivation is effected by the egg or by something operating in the sperm receptacles or ducts of the female before the sperms reach the eggs. Many gynandromorphs and other sex mosaics are found in this material, but their presence may be accounted for

about equally well on either assumption. Likewise, since it is the zygotic constitution of the female which is responsible for the sex of her total progeny, the selective influence might act in one organ or region as well as another.

As to the validity of the original premises (that both X-bearing and Y-bearing sperms are produced by each male) it may be noted that since the preceding paper on this subject was written further studies have been made on the mutant character there considered, particularly in crosses with a "bisexual" strain recently secured. Also a second sex linked character in this species has been found and similarly studied (unpublished data). These studies fully support the earlier evidence and seem to leave no question on this point.

It is felt that the numerous genetic and cytological peculiarities exhibited by *Sciara* make it desirable to use especial caution in drawing conclusions here. For this reason comparisons and further discussion may be omitted for the present, even though the inferences outlined above appear to be warranted by the evidence as it stands.

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LINKAGE STUDIES OF FACTOR PAIRS FOR NORMAL *VS.* GLOSSY SEEDLINGS AND FLINTY *VS.* FLOURY ENDOSPERM IN MAIZE[1]

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STUDIES of linkage groups in maize have as their immediate purpose the location of factors in specific chromosomes and the determination of the number of linkage groups. Such information will furnish a foundation for the study of the factor relations of the more complex characters. It is important, also, to learn the extent to which the laws of factor relations and chromosome mapping which have been worked out for *Drosophila melanogaster* will hold in other organisms. In so far as can be determined, the data regarding linkage relations in maize can be explained in the same manner as has been used for Drosophila. With peas, however, Wellensiek (1928) presented data which he explained on the basis of more than 50 per cent. crossing over. With Oenothera, the cytological and genetic results lead to the hypothesis that the number of linkage groups may be very different from the number of chromosome pairs.

In a previous paper (Hayes and Brewbaker, 1928) it was pointed out that there were at least three independently inherited factors for glossy seedlings, and these were called gl_1, gl_2 and gl_3. It was learned also that the factor pairs for flinty-floury and for glossy seedling, Gl_2gl_2, were linked and were independent in inheritance of certain other factor pairs.

The factor symbols *C* and *F* were used by Hayes and East (1915) for flinty and floury, respectively. In this paper, *Fl* is used for flinty and *fl* for floury endosperm,

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although flinty is not dominant to floury. Flinty and floury are endosperm characters and are dependent upon double fertilization. Two doses of either factor are dominant to one dose of its allelomorph. Thus, the appearance of the seed is a direct indication of the factor condition of the female gamete.

The purpose of the present paper is to give further facts regarding the linkage relations of *Flfl* and Gl_2gl_2. The factor pairs, *Flfl* and Gl_2gl_2, were studied in relation to one or more factors in each of the well-known linkage groups. An alphabetic list of the factor symbols, with a brief statement of the character, will be given. The publication reference for all characters listed, except slashed, may be obtained from Hayes and Garber (1927). A description of slashed and its linkage relations will be reported in a separate paper by one of the writers. In the following list dominant factors are capitalized.

A	anthocyanin, general plant color found in aleurone, pericarp, stems, leaves, etc.
br	brachytic, characterized by shortened internodes
Bn	brown aleurone
C	colored aleurone, complementary to *A* and *R*
g	golden plant color
lg	liguleless leaf
Pr	purple aleurone
ra	ramose ear
sh	shrunken endosperm
sl	slashed or shredded seedling
su	sugary endosperm
Tu	tunicate ear
wx	waxy endosperm
Y	yellow endosperm

LINKAGE RELATIONS OF *Flfl* AND Gl_2gl_2

In a previous study, F_2 data were presented for three different crosses for linkage relations of *Flfl* and Gl_2gl_2. The results were as follows:

No. individuals	Crossover value
1186	.309
874	.303
260	.331

Another F_2 generation was studied recently from a cross of $Flgl_2 \times flGl_2$. The results were as follows: $FlGl_2$, 357; $Flgl_2$, 163; $flgl_2$, 421, and $flgl_2$, 81. The linkage value was calculated from the formula (Collins, 1924): $p = \frac{2(AB + ab)}{n} - .5$, where AB and ab are the new combinations of parental characters. The calculated value of p, or crossing over expressed as a decimal fraction, was .357. Linkage relations of *Flfl* and Gl_2gl_2 were studied also in a backcross of *Flfl* Gl_2gl_2 with *FlFl* gl_2gl_2. The F_1 was used as the female parent. The results were as follows:

$FlGl_2$	$Flgl_2$	$flGl_2$	$flgl_2$
175	388	426	139

The crossover percentage was 27.8.

The factor pair Gl_2gl_2 was located in the "B" group on the basis of linkage relations with *Lglg*. The linkage relations obtained from F_2 data in the repulsion phase follow:

Gl_2Lg	Gl_2lg	gl_2Lg	gl_2lg
536	279	290	6

Letting the four phenotypic groups be represented by a, b, c and d, and using the formulas and calculated values given by Fisher and Balmukand (1928), $\frac{ad}{bc}$ for repulsion is equal to .03975 which is equal to a p value of .138. Probable errors were computed from tables prepared by Dr. F. R. Immer, of this division. The computed P. E. is .020. The C. O. percentage, then, is 13.8 ± 2.0.

The factor pair *Flfl* was studied also in relation to *Lglg* in a cross of pale yellow, floury endosperm, normal plant with yellow, flinty endosperm, liguleless plant. Segregation was obtained from a greenhouse planting of all the yellow kernels. Seventy-seven pale yellow and ninety colorless seeds were retained to plant in the field for the

purpose of learning the accuracy of the classification for pale yellow and white. This was about one-third of the pale yellow seeds and one-half of the colorless. The segregation of this group was calculated on the basis of the results obtained from the remainder of the seeds which were planted in the greenhouse. The F_2 data, after making the above corrections, are as follows:

Flinty		Floury	
Lg	*lg*	*Lg*	*lg*
691	230	628	192

Using the formula $p = \frac{2(AB + ab)}{n} - .5$ with AB and ab containing the new combinations of parental characters, the calculated value of p becomes .514.

Calculated crossover values of *fl* and gl_2 range from 27.8 to 35.7 per cent., and gl_2 and *lg* gave a calculated linkage value of 13.8 ± 2.0. The crossover per cent. of 51.4 was obtained for *lg* and *fl*. These data indicate that *fl* and gl_2 are on the same side of *lg* in the chromosome, and that *fl* is so far removed from *lg* that the crossover value would have indicated independent inheritance had it not been for the linkage already demonstrated of gl_2 with *fl* and *lg*. These relationships will be substantiated by studies of backcrosses or by F_2 studies in the coupling phase. In the meantime, the data are given so that others can use these characters, flinty-floury and glossy$_2$, in studies of linkage relations with particular reference to the "B" linkage group to which they belong. Flinty-floury can be differentiated by examination of the kernels, while glossy can be differentiated from normal by growing the seedlings in the greenhouse. For these reasons, they are very desirable characters for linkage study.

INDEPENDENT INHERITANCE OF Gl_2gl_2 AND OTHER FACTOR PAIRS

In a previous paper, it was shown that Gl_2gl_2 probably was independent in inheritance of the aleurone factor pairs *Rr*, *Cc* and *Aa*. Further F_2 data for linkage relations of Gl_2gl_2 and other characters are given in Table I.

TABLE I

SUMMARY OF STUDIES OF LINKAGE RELATIONS OF Gl_2gl_2 AND OTHER CHARACTERS. F_2 DATA.

Character	Culture No.	Coupling or Repulsion	A^*Gl_2	Agl_2	aGl_2	agl_2	p
Prpr	H167, 169, 170, 176, 179	R	3404	1236	1506	488	.484 ± .006
Susu	H181 (1928)	C	372	128	78	22	.528 ± .020
Tutu	H182 × H27	backcross	138	113	122	93	.504 ± .016
Brbr	181 (1928)	R	364	115	86	35	.535 ± .020
Brbr	174 (1928)	R	610	177	169	57	.521 ± .016
Yy (Long-fellow)	168, 175 (1928)	R	1202	369	364	119	.509 ± .011
Gg	168 (1928)	R	610	184	168	60	.524 ± .015

* In this table *A* and *a* represent the dominant and recessive character, respectively.

Crossover values and their probable errors were calculated by the product method which has been described previously. Extensive data for the factor pairs *Prpr* and *Flfl* were available. The calculated value of p was $.484 \pm .006$. The deviation from 50 per cent. crossing over or independent inheritance is about three times the probable error. The p values for linkage relations of Gl_2gl_2 with various other factor pairs indicate that the deviations from independent inheritance are within expectation, as determined by the probable errors.

INDEPENDENT INHERITANCE OF FLINTY-FLOURY WITH OTHER CHARACTERS

Flinty-floury segregates in a 1:1 ratio on the ears of F_1 plants. It has been shown that the character observed is an expression of the genotype of the female parent (Hayes and East, 1915). It is the best illustration available of the dominance of two doses of a factor over one dose of the opposite factor, and *vice versa.*

In the studies given here of the relation of flinty-floury and other characters, X^2 was used for testing independent inheritance (Collins, 1924). For this purpose, the theoretical value is calculated on the basis of the observed ratio for each pair of characters with the added hypothesis that the characters segregate independently of each other.

The method can be used for a test of independence of more than two character pairs. In a cross of sweet with floury, the F_1 was colored aleurone flinty, and in F_2, segregation for *Cc, Rr, Flfl* and *Susu* occurred. The following results are compared with those calculated on the basis of independent inheritance:

		Obs.	Cal.	$(O-C)^2/C$
Flinty	Colored	784	804.35	.51
"	Colorless	654	628.78	1.01
Floury	Colored	775	757.49	.40
"	Colorless	571	592.16	.74
Sweet	Colored	470	466.52	.03
"	Colorless	360	364.70	.06

$n = 3$. $X^2 = 2.75$. $P = .2593$

As bad or worse fit might be expected on the basis of random sampling 25.9 times out of 100. These results are in agreement with those of a previous study which indicated that *Rr* and *Cc* are independent in inheritance of Gl_2gl_2. The factor pair *Susu* also appears independent in inheritance of *Flfl*.

The F_2 data for other characters in relation to flinty-floury are given in Table II, together with X^2 for independence and calculated *P* values.

TABLE II

SUMMARY OF DATA ON FLINTY-FLOURY *vs.* OTHER CHARACTERS ON THE BASIS OF X^2 FOR INDEPENDENCE

Culture No.	Cross	Flinty		Floury		X^2	*P*
		A	*a*	*A*	*a*		
H84, H85	Flinty colored aleurone × floury "R" tester	1517	556	1450	413	11.43	.0007
H146, H168, H170	Flinty normal × floury golden plant	1153	336	1082	301	.27	very large
H157, H173, H176	Flinty, No. 13 yellow endosperm × floury colorless	2304	793	2296	862	2.31	.1344
H168	Flinty yellow in Longfellow × floury colorless	367	153	390	112	6.75	.0097
H173	Flinty slashed seedling × floury normal	1007	270	983	274	.16	very large
13b, H173	Flinty gl_1 × floury normal seedling	1528	553	1378	532	.83	very large
H170	Flinty sorghum tassel × floury normal	526	110	457	124	3.20	.0757
H84	Flinty red aleurone × floury "R" tester	524	236	502	238	.20	very large
H157	Floury ramose × flinty normal	133	51	117	43	.02	very large
H157	Flinty virescent × floury normal	359	110	351	116	.24	very large

In the study of a cross of flinty colored aleurone × "R" tester floury, X^2 for independence was 11.43 and P .0007. As bad or worse result might be expected on the basis of random sampling approximately once in every 1,400 trials. This indicates a poor fit. The seeds in this cross were small and the separation somewhat difficult for flinty *vs.* floury endosperm. Many of the seeds were cut to aid in the separation. The deviation from expectation is such that, if linkage were responsible for this large deviation from expectation, more than 50 per cent. crossing over occurred.

For the F_2 culture, H168, for *Flfl* and *Yy*, yellow endosperm from Longfellow, a X^2 of 6.75 and P value of .0097 were obtained. As bad or worse fit might be expected on the basis of random sampling approximately once in 111 trials. The factor *Y* obtained from Minn. No. 13 was also used in cultures H157, H176, to determine the relation of *Yy* to *Flfl*. In this case, a P value of .1344 was obtained which is an indication of independent inheritance.

The F_2 data for other character pairs indicate independent inheritance of *Flfl* and *Gg* of the "R" group and *Slsl*, Gl_1gl_1 and *Rara* of the "Bn" group. The data for culture H84 give the segregation obtained for flinty-floury and purple *vs.* red aleurone. The X^2 is .20 and P very large which indicates independent inheritance. Flinty-floury is probably independent in inheritance of normal *vs.* sorghum tassel (see Hayes and Brewbaker, 1928) with a X^2 value for independence of 3.20 and a value of P of .0757. Flinty-floury appears independent, also, of normal *vs.* virescent seedling, a character pair obtained from Minn. No. 13 which has not been placed in any linkage group.

SUMMARY OF RESULTS

(1) Flinty *vs.* floury endosperm segregates in a ratio of 1:1 on the ears of F_1 plants. As a rule, there is a slight excess of flinty over floury seeds. Data are

given for the linkage relations of *Flfl* and Gl_2gl_2 with factors in known linkage groups. Brief summaries of the results of previous studies are included.

(2) Further data were presented in this paper for the linkage relations of *Flfl* with Gl_2gl_2, a factor pair for normal *vs.* glossy seedlings. F_2 data gave crossover values of .309, .303, .331 and .357. From a backcross of *Flfl* Gl_2gl_2 with *Flfl* gl_2gl_2, the calculated crossover percentage of 27.8 was obtained.

(3) Linkage relations were studied from F_2 data of crosses of Gl_2gl_2 and *Flfl* with factor pairs in known linkage groups. The factors for glossy seedling gl_2 and for liguleless *lg* gave a calculated crossover value of $.13 \pm .02$. From a total of 1,324 F_2 individuals from a cross of flinty liguleless with floury normal, the calculated recombination value of .514 was obtained. The order of the genes appears to be $fl - gl_2 - lg$.

(4) There appears to be independent inheritance between Gl_2gl_2 and the *Cc* aleurone factor pair, the *Wxwx* factor pair for starchy *vs.* waxy endosperm, and the *Shsh* factor pair for normal *vs.* shrunken endosperm of the "C" linkage group. Independence of *Flfl* with the *Cc* factor pair of the "C" linkage group is also shown.

(5) The factor pairs Gl_2gl_2 and *Flfl* appear independent in inheritance of *Gg*, the factor pair for normal *vs.* golden plant, and *Rr*, the aleurone factor pair of the "R" group, and of the factor pairs, *Susu* and *Tutu*, starchy *vs.* sugary, and tunicate *vs.* normal of the "su" group. Gl_2gl_2 and *Flfl* appear independent in inheritance of *Yy*, the factor pair for yellow *vs.* colorless endosperm of the "Y" group.

(6) Two separate studies have been made of the linkage relations of Gl_2gl_2 and *Brbr*, the factor pair for normal *vs.* brachytic plant of the "P" group. Recombination values of $.535 \pm .020$ and $.521 \pm .016$

were obtained. Linkage relations of Gl_2gl_2 and *Aa*, the aleurone factor pair, were studied in the F_2 of a cross where both *Aa* and *Rr* were segregating. From a total of 2,147 individuals, the calculated value of p was .504 which indicates independent inheritance.

(7) The X^2 test was used as a measure of independence to determine the relation of *Flfl* with Gl_1gl_1, a factor pair for normal *vs.* glossy seedlings; *Slsl*, a factor pair for normal *vs.* slashed seedlings, and *Rara*, the factor pair for normal *vs.* ramose tassel of the "Bn" linkage group. The value of P was very large in all three cases indicating independent inheritance.

(8) Flinty-floury appears to be independent in inheritance of factor pairs *Sgsg*, normal *vs.* sorghum tassel, and normal *vs.* virescent seedling. These two factor pairs have been studied extensively in relation to factors in known linkage groups without discovering a linkage relation. Flinty-floury also appears independent in inheritance of *Prpr*, the purple aleurone factor pair.

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INFLUENCE OF DESICCATED THYROID AND IODINE ON GROWTH—II. WITH A STANDARD ACID DIET[1]

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PRELIMINARY to a study of the influence of avitaminoses on the internal secretions and thus on embryonic and postnatal growth, experiments were run in which iodine was added to acid and basic diets. The experiments here described are those in which on standard *acid* diet iodine was exhibited in the presence of glucose.

The rats used were kept in cages eighteen inches long by sixteen inches wide by eight inches high at the back, sloped to five inches at the front. They were made of one half inch wire mesh, the cages being set in galvanized iron pans to facilitate cleaning. The top of the cage was sloped to allow a water bottle to lie on it, furnishing a continuous supply of distilled water at all times. The animals were kept out of drafts, receiving sunlight only through glass for about three hours daily.

Experiment I: Mature rats. The animals used in this experiment were pink-eyed yellow rats, inbred for eight generations, approximately six months of age at the time the experiment was begun. They were confined in the wire cages described above, and were fed at night, in order to ensure the complete consumption of their rations.

The maternal diet for several generations had consisted of two parts yellow corn; one part wheat bran; one part oats; one part wheat middlings; one part tankage and one half part linseed oil. City water had been furnished in siphon bottles.

Eight female rats and four males, all approximately six months of age, were divided into four lots, with two

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females and one male in each lot. Weights and records were taken at intervals of five days.

Lot I: Acid food mixture. (Females Nos. 22, 31; male No. 81). Three parts oats, one part corn, one part wheat bran, fed in quantity of eighty grams each night. Distilled water was added for mixing.

Lot II: Acid food mixture, eighty grams, plus two grains of desiccated thyroid, daily. The desiccated thyroid was added to the acid mixture, then distilled water added and the mixture stirred thoroughly. Females Nos. 40, 57; male No. 94.

Lot III: Acid food mixture, eighty grams, plus forty-five grams of a 50 per cent. glucose solution (white Karo corn syrup in distilled water). Females Nos. 41, 74; male No. 86.

Lot IV: Acid food mixture, eighty grams, plus forty-five grams of 50 per cent. glucose; plus two grains of desiccated thyroid. Females Nos. 47, 48; male No. 84.

In lots III and IV the glucose mixtures were prepared each day, as otherwise they spoiled. Distilled water was available at all times in siphon bottles.

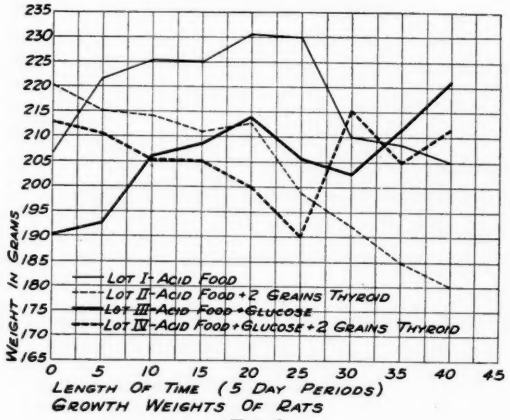

FIG. 1.

In every lot at least one female became pregnant and produced young. In Lot I the food apparently main-

tained weights, inducing a gain until during the lactation period (both the females littered) there was a slight decrease in weight. In Lot II there was a gradual loss in weight due to the thyroid dosage. Female No. 40 had a litter of six young, so weak that they died in two days. In Lot III there was a steady increase in weight until lactation. Female No. 74 died just after she had littered. Female No. 41 produced six young and reared them together with three from the deceased No. 74.

In Lot IV both females conceived, but in the case of No. 48, the ten young died shortly after birth, while No. 47 died while littering. In both the females and the male there was a slight loss of weight. The iodine dosage was not sufficient to prevent pregnancy or to cause the resorption of the young, but was apparently injurious to the mothers and their offspring.

Experiment II: Young rats. In this experiment young rats, closely related since their parents were all litter mates, were selected from three litters that totaled twenty-three young. They were all born on the same day and were two months and three days old when they were placed on the experimental diet. They were confined in wire cages as described elsewhere, and were fed at night.

The maternal and after-weaning diet of the rats had been a mixed one, consisting of yellow corn, wheat bran, oats, wheat middlings, tankage and linseed meal, as described above, with a plentiful supply of city water available in siphon bottles. This mixed ration was the "complete ration" furnished all the colony for many generations.

Sixteen animals were used, eight females and eight males. Females and males of the same litters were distributed throughout the lots so far as possible. Weights and records were taken at the beginning of the experiment and subsequently at intervals of five days.

Lot I: Acid food mixture. Seventy grams of a mixture of three parts oats, one part yellow corn and one part

wheat bran was furnished daily in a dry condition. Distilled water was at all times available in siphon bottles. Females Nos. 1 and 2; males Nos. 3 and 4.

Lot II: Acid food mixture and desiccated thyroid. Seventy grams of the mixture of oats, yellow corn and wheat bran had added to it one grain of thyroid daily, with distilled water utilized to moisten thoroughly. Females Nos. 5 and 6; males Nos. 7 and 8.

Lot III: Acid food mixture with glucose. Seventy grams of the acid mixture were mixed with thirty-five grams of a 50 per cent. solution of glucose. (One half white Karo corn syrup and one half distilled water.) Females Nos. 9 and 10; males Nos. 11 and 12.

Lot IV: Acid food mixture, glucose and the desiccated thyroid. To seventy grams of the acid food mixture, thirty-five grams of a 50 per cent. glucose mixture and one grain of thyroid were added each day. Females Nos. 13 and 14; males Nos. 15 and 16.

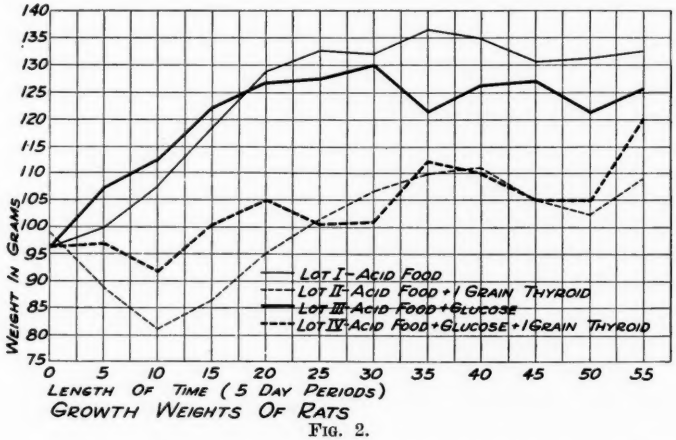

FIG. 2.

As shown (Figure 2), the animals in Lots I and III gained steadily from the beginning of the experiment, and Lot I continued to hold a high average throughout the duration of the experiment. On the other hand, those

in Lot III showed a decided decrease in weight at about the thirty-day period. This was occasioned by the birth of the young and the drain on the system accompanying lactation. It is important to note also that Lot III, which received a glucose diet, gave birth to young when the females were quite young, only ninety-five and ninety-six days old. Since the vagina of the domestic rat does not open until the animal is seventy-two days of age (Donaldson, H. H., "The Rat," pp. 192) and the gestation period is approximately twenty-three days, it will be noted that these females mated very soon after the vagina opened. It is an unusual circumstance that any of the young were saved, as the rats of this age are generally unable to provide a sufficient milk supply for their young. The females of Lot I produced no offspring and were never pregnant.

Lot II lost rapidly at the beginning of the experiment but soon began to gain. They always showed a craving for their food and consumed it completely. The animals of this lot tended to leanness after the tenth day and developed extreme nervousness. The females did not become pregnant.

Lot IV, receiving both *glucose* and *thyroid,* showed a gradual gain in weight but exhibited no signs of pregnancy. All the lot except one male died before the end of the experiment.

Experiment III: Mature rabbits. Six female rabbits averaging fifteen months of age, with records of fertility, were placed in separate cages and given experimental diets. They were mated from time to time with a young male known to be fertile.

The basic portion of the standard diet included green lettuce and celery with clover and timothy hay and occasional lots of cabbage and turnips. The acid mixture consisted of three parts oats, one part bran, one part yellow corn, with coarse ice-cream salt added at each mixture.

Lot I: Acid food mixture. Females Nos. 16 and 17. Acid mixture consisted of three parts oats, one part

yellow corn, one part wheat bran, fed dry in quantity of one half pint per day for each animal. A small quantity of salt was mixed with the food and distilled water was constantly available in drinking dishes.

Lot II: Acid food mixture with glucose. Females Nos. 1 and 13. To the acid food mixture, enough 50 per cent. glucose (white Karo corn syrup in distilled water) was added to make a quart of the mash for each individual. Distilled water was used for drinking.

Lot III: Acid food mixture plus glucose plus KI. Females Nos. 4 and 12. To the acid mixture with 50 per cent. glucose, was added six grains of KI for each animal.

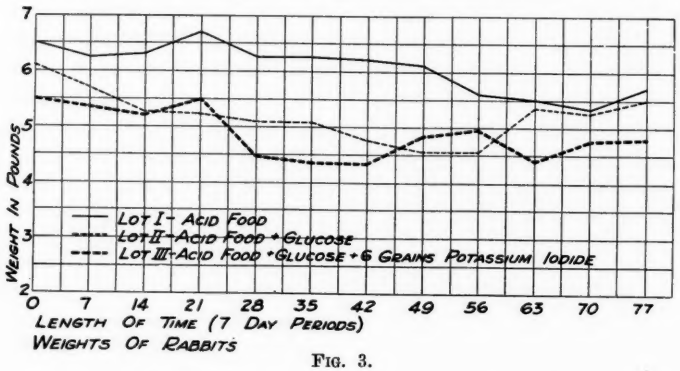
WEIGHT IN POUNDS
7
6
5
4
3
2
LOT I - ACID FOOD
LOT II - ACID FOOD + GLUCOSE
LOT III - ACID FOOD + GLUCOSE + 6 GRAINS POTASSIUM IODIDE
0 7 14 21 28 35 42 49 56 63 70 77
LENGTH OF TIME (7 DAY PERIODS)
WEIGHTS OF RABBITS

FIG. 3.

Lot I: Acid food. In this experiment there was a loss in weight due to lack of green vegetables. The vitamin content was limited to vitamins A and B.

Lot II: Acid food with glucose. In this lot, No. 13 littered but devoured her young. In No. 1 the glucose apparently induced an emaciated condition, and after one month of treatment the animal was furnished ten drops of cod liver oil per diem, with green vegetables for a period of eleven days, when she was transferred to the ration again.

Lot III: Acid food, glucose, with KI. One female, No. 12, littered on July 15, but ate her litter. The stock were all subject to respiratory troubles (snuffles) indi-

cating perhaps a deficiency diet. The glucose seemed to stimulate the appetite at first, but the animals soon lost their interest in the food. It is not believed that the rabbit experiments were particularly significant on account of the general poor health of the stock.

Cod liver oil proved very beneficial in *snuffles* and in several cases restored the animals to health.

General Conclusions

In the mature rats it was evident that the food was adequate for reproduction, in spite of the fact that it did not contain green vegetables. However, in all probability there was enough vitamin E from the wheat.

In lots without glucose, the diet, especially in vitamins A and B, proved adequate for normal growth and reproduction. The other lots received diets extremely rich in vitamin B and were given an increased caloric value by the addition of glucose.

The necessary calcium, phosphorus and sodium were supplied, but the requisite *iron* ordinarily obtained from green vegetables was lacking.

In a study of the laxative action of wheat bran, Williams (1926) discovered that the crude fiber was not utilized by dogs but acted as a laxative more powerful than powdered agar. On the other hand, Porter (1924) has concluded that bran is not an intestinal stimulant just because it is coarse. He cites the fact that the wheat berry, as grown here in America, contains 19.7 per cent. of chemical elements. The flour contains 1.75 per cent. and the bran the remainder. His conclusion is that intestinal stasis is really *avitaminosis.*

It seems evident that regardless of whether wheat bran furnished the necessary vitamin material, oats and yellow corn gave sufficient vitamin A.

In the young rats glucose hastened maturity, as in Lot III the females produced litters when only ninety-five and ninety-six days old.

In normal animals, thyroid feeding diminishes carbohydrate tolerance, raises blood sugar, and leads to great reduction in the carbohydrate content of the liver.

Reid Hunt (1910) has pointed out that diets such as dextrose and liver greatly increase resistance of mice to acetonitrile, while eggs, milk, cheese and various fats lower such resistance. He compares the dextrose and liver with thyroid.

It should be pointed out that liver contains iron, iodine and in fact is rich in all the minerals that are carried in the blood. Since it also is rich in glucose, it undoubtedly furnishes proper material for the process of sugar decomposition that in the animals is the reverse of synthesization in plants.

In our experiment, we added carbohydrate at the same time that we furnished thyroid, which definitely reduced the carbohydrate content of the liver.

In the light of the recent discovery that vitamin B contains *inosite,* which has the chemical composition of glucose, it seems quite likely that we were not only adding the requisite sugar but were furnishing an *abundance of* one of the so-called *"vitamins."*

In our experiments thyroid feeding quite obviously speeded metabolism and in the case of thyroid in the presence of glucose, katabolism was tremendously increased. It is possible that the excess of glucose furnished greater caloric energy, which was utilized in the presence of the thyroid. That low dosage of thyroid induces not only weight increase, but elongation of bones, has been shown more conclusively in experiments with chicks to be recorded elsewhere.

SUMMARY

(1) After preliminary feeding with a mixed diet, rats and rabbits were furnished a standard acid diet consisting of yellow corn, bran and oats, while certain lots were fed glucose, with and without the addition of desiccated thyroid extract.

(2) In the mature rats, pregnancies appeared in all lots. Thyroid dosage induced injury to the young during

lactation, but did not inhibit pregnancy nor cause resorption of the young.

(3) In the rabbits, the experiments are not particularly significant on account of snuffles affecting the stock.

(4) In young rats thyroid with glucose produced steady gain in weight, while with glucose alone, maturity was stimulated and early pregnancies resulted.

(5) Low thyroid dosage induced growth of the bones, with increased weights.

(6) The addition of glucose to the diet containing iodine is particularly interesting in the light of the recent discovery that inosite has the structural formula of *glucose.*

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THE NATURAL HISTORY OF CLADOCERANS IN RELATION TO TEMPERATURE

I. DISTRIBUTION AND THE TEMPERATURE LIMITS FOR VITAL ACTIVITIES

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I

AN attempt[1] to analyze the relation of temperature to the biology of closely related species of animals permits of several lines of attack. The natural conditions of existence are the foundation upon which the study is built; the data derived from experimentally imposed conditions must be superimposed upon this foundation of seasonal rhythm and geographical range. For certain animals, temperature can be regarded as an important factor controlling their range and periods of abundance. An investigation of a problem, like this one at hand, then, would necessitate a preliminary knowledge of the habitats of the species used, the upper and lower temperature limits for both vital and reproductive activity, the effect of temperature on the speed of development and the correlating of these three lines of attack into a consistent whole. This plan has been followed in attempting to ascertain the rôle played by temperature in the ethology of a sharply delimited group of Entomostraca, the Cladocera, and in this first paper we will consider the distribution and seasonal cycles for several common species and their upper and lower temperature limits.

II

That cladocerans are cosmopolitan in distribution is a generally accepted statement. A list of species from one region is typical of that from many other regions.[2] This

[1] The data given in this paper are results of work done at the Zoological Laboratory, Harvard University, and the Department of Genetics, Carnegie Institution of Washington.

[2] Beardsley, 1902; Birge, 1878, 1891, 1893, 1897, 1910; Herrick, 1895; Coker, 1926; Juday, 1902, 1920; Stromsten, 1920; and many others in this country and many in Europe.

is true to a large degree, but it is also true that some species are restricted to a particular region. Cladocerans are also restricted in another sense. They do not occur in equal numbers in the same region throughout the year, but appear in rhythms or cycles. At certain

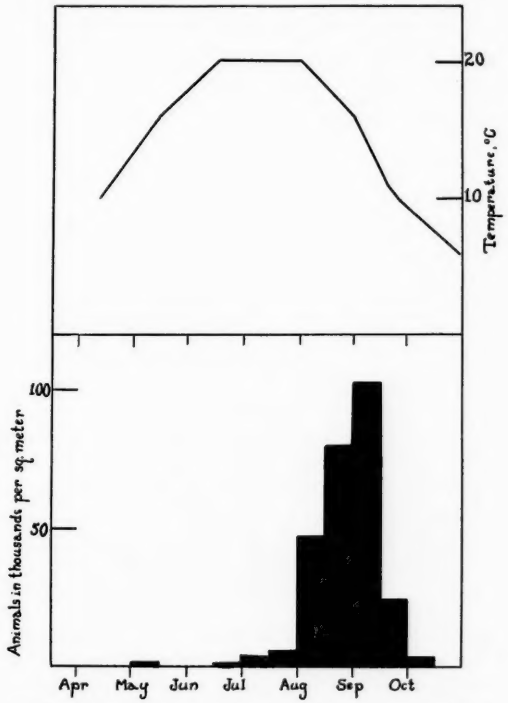

FIG. 1. Annual rhythm of abundance of *Sida crystallina* in thousands of animals per square meter of surface for Dobersdorfer See from April to November 1891 (Apstein, 1896). The upper graph shows the temperatures of the water for the same months.

times of the year a given species may populate a lake or pond with thousands of individuals per square foot of surface and a few weeks later be represented by but a few. As it is not within the scope of this paper to consider the complete evidence for the geographical range

and seasonal rhythm of each species used, the following condensed account is given. The data are taken from the collections of the writer and from unpublished field notes loaned by Dr. A. M. Banta. These data were collected over a period of several years and were further amplified and checked by reference to the following papers: Apstein, 1898; Baird, 1850; Beardsley, 1902; Birge, 1878, 1891, 1897, 1910, 1918; Coker, 1926; Ekman, 1905; Green, 1919; Herrick, 1884, 1895; Juday, 1920; Murray, 1911; Stingelin, 1895; Wesenberg-Lund, 1908.

Family SIDIDAE

Sida crystallina (O. F. Müller) 1785

Range: This species is probably circumpolar in the northern hemisphere. It may occur in the southern states, but no records of its occurrence there are at hand.

Annual rhythm: The most complete records are given by Apstein 1896. (See Fig. 1.) This species seems to be most abundant in this country in October and November, although it can be taken during the late spring and summer.

Pseudosida bidentata Herrick 1884

Range: Described from the Gulf States and known to occur in Ceylon, Siam, Sumatra, northern South America.

Annual rhythm: Known to occur during the winter and spring, but the complete cycle is not known.

Latanopsis occidentalis Birge 1891

Range: Usually found in Florida and west through the Gulf States, and is only sporadic in the north.

Annual rhythm: The rhythm is not known, except that it usually disappears from the ponds in the north by September.

Family DAPHNIDAE

Daphnia magna Straus 1820

Range: This form is widespread throughout North America and Europe.

Annual rhythm: Not known to the writer.

Daphnia pulex (de Geer) 1778

Range: This species is undoubtedly the most common and widespread of all the larger cladocerans. It has been reported from a latitude of 82° North in Greenland and as far south as the Falkland Islands and Tierra del Fuego.

Annual rhythm: Figs. 2 and 3 show typical seasonal distributions of this

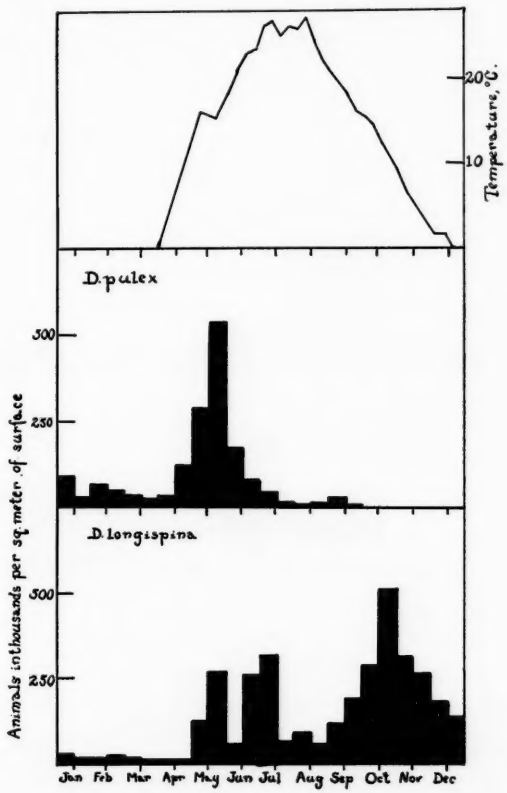

FIG. 2. Annual rhythms of occurence for *Daphnia pulex* and *Daphnia longispina* in thousands of animals per square meter of surface for Lake Mendota in 1896 (Birge, 1897). The upper chart gives the temperature of the water for the same year.

common species. It will be noted that it is a form that occurs in maximum numbers in the spring and fall.

Daphnia longispina (O. F. Müller) 1785

Range: This species is as widespread but not as common as *D. pulex*.

Annual rhythm: This species may be found in every month of the year as is shown by Figs. 2 and 3, but it is most abundant in spring and fall and occasionally in the winter.

Scapholeberis mucronata (O. F. Müller) 1785

Range: It is known to occur in the central and southern states and has been reported from many places.

Annual rhythm: This species is known to occur in New York and Massachusetts from April to November and has been taken when the temperature of the water was 5° C. but not enough data are available to give maxima and minima of abundance.

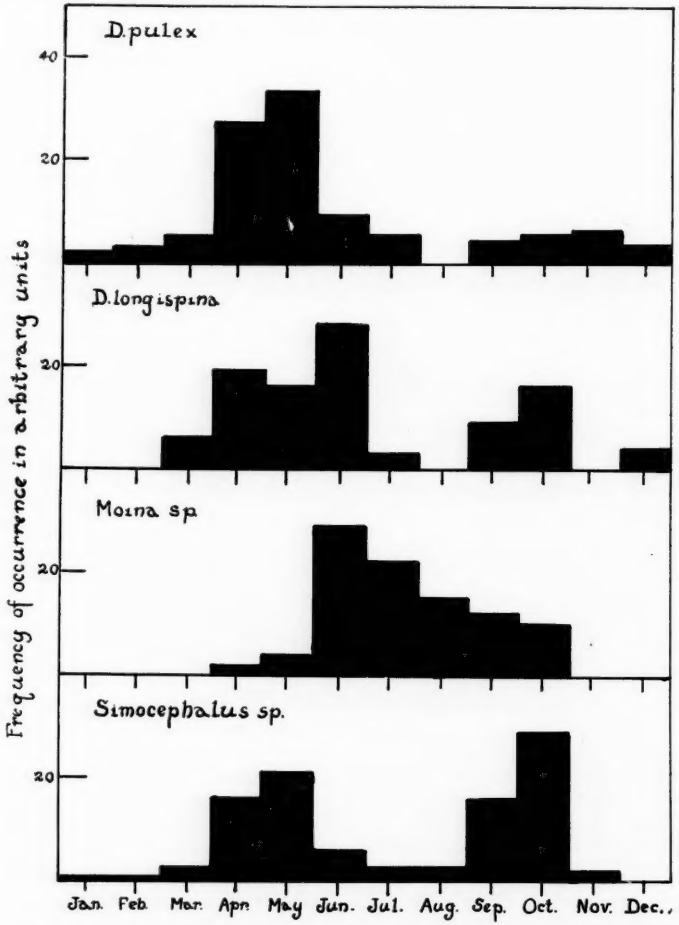

FIG. 3. Original data for the occurrence of *Daphnia pulex, Daphnia longispina,* genus *Moina* and the genus *Simocephalus.* The graphs shown comprise the accumulated data for several years' collections in different parts of eastern United States, but chiefly for Long Island and Massachusetts. The frequency of occurrence is expressed in arbitrary units and so adjusted that the areas of the graphs are the same for each form.

Simocephalus Schoedler 1858

There are three common species of this genus found in the United States and as their occurrence is similar they will be considered together.

Range: This genus is widely distributed, having been collected wherever other cladocerans occur. It is a bottom feeder and often escapes detection in towings and surface samples.

Annual rhythm: This form can be found in every month of the year (Fig. 3). There are two maxima of abundance, one in April and May, when the temperature of the water varies between 14° and 20° C., and one in September and November, when the temperature of the water is near 15° C.

Ceriodaphnia laticaudata P. E. Müller 1867

Range: Probably of general occurrence throughout the United States.

Annual rhythm: All available data seem to show that this species occurs throughout the year with probable maxima in April and October.

Moina Baird 1850

There are several species of this genus, and as there is often doubt as to the proper identification they have been confused in faunal lists. This is due to the fact that proper identification can only be determined from the ephippial females or the males and these are not always present in collections.

Range: Widely distributed throughout North America and Europe in small muddy pools.

Annual rhythm: There is ample evidence to show that this genus is found principally during the summer months. (See Fig. 3.)

Family MACROTHRICIDAE

Macrothrix rosea (Jurine) 1820

Range: Seldom mentioned in faunal lists from the northern states but definitely known to occur in Florida.

Annual rhythm: Stingelin (1895) speaks of it as rare and mentioned its occurrence in the summers of 1893 and 1894.

The cladocerans here listed may be divided into three groups according to their geographical distribution. The first and largest group includes those which are found over a wide range of territory and can not be considered as being restricted. This group includes *Daphnia pulex, D. longispina,* all the species of *Simocephalus, Scapholeberis mucronata, Ceriodaphnia laticaudata* and probably the species of *Moina.* The second group includes two species, *D. magna* and *Sida crystallina,* which it seems likely are northern in distribution. The third group consists of those whose habitat is the southern states. In this group are certainly *Pseudosida bidentata*

and *Latanopsis occidentalis* and probably *Macrothrix rosea.*

Of the species which have wide distribution the seasonal rhythms of the northern clones are known. With the exception of the species of *Moina,* which occur in greatest numbers in the warmest part of the summer in shallow exposed pools, these species have maxima of abundance in the spring or in the fall or in both spring and fall, when the temperature of the water they inhabit is between 10° and 20° Centigrade. In addition to these species, *Sida crystallina* is known to have a fall maximum when the temperature of the water in which it lives is declining.

III

While the density of population for any one of a given group of closely related species depends to a large extent on the speed of development and on the frequency of the production of young after the animal becomes adult, the presence or absence of a species is limited by the maximum and minimum temperatures which the animals are able to endure. This check by high or low temperatures may apply equally to geographical range or to seasonal rhythm. Since we have seen that cladocerans show differences in season and in range of occurrence it is of interest to see if these differences are in any way correlated with the effects of high and low temperatures experimentally imposed in the laboratory.

Much of the work with low temperatures has been done by plant physiologists in trying to understand the factors involved in cold-hardiness. In plants death from freezing has been considered due to the mechanical injury from the formation of ice crystals within the cells, to the loss of water from the tissues or (what is more probable) to the precipitation of proteins or some other chemical effect which is irreversible. Semper (1881) in his book on "Animal Life" remarks on the effects of low temperatures on animals and he used the term "chill-coma" to denote the inactivity of animals when subjected to low

temperatures. He notes the fact that the critical temperature for "chill-coma" is widely different in various animals and even among animals living in fresh water. An account of the effects of low temperature on insects was given by Bachmetjew (1901). He called attention to the fact that the time rate of cooling is very important and determines the point at which "chill-coma" will supervene. If the temperature of an animal is lowered quite rapidly this critical point is reached at a higher temperature than if the cooling is done slowly. A review of the literature on the freezing and survival of insects is given by Payne (1926). Her own work concerned the relative effect of low temperature on three groups of insects that belonged to distinct habitats. The aquatic insects were found to be affected at a higher temperature than were the oak-borers, which are subjected in nature to great extremes of temperature. She also found in the latter animals a decided periodicity in their hardiness. Ackerman (1926), working with the grain aphid, found that the previous "habitat" temperature at which the animal had been reared controlled the temperature at which the fat (lipoid) globules of the animals would solidify. In my own experiments I have failed to find any correlation between the temperature at which the daphnids were reared and the length of time necessary for inactivation at low temperatures. This is in agreement with the observation of Payne on aquatic insects.

In the experiments on inactivation by low temperatures adults of the following species were used: *Pseudosida bidentata, Moina macrocopa, Simocephalus vetulus, S. serrulatus, Daphnia pulex* and *D. longispina.* The method was as follows. A cake of natural ice was placed in a glass container on the stage of a binocular dissecting microscope. A small cylindrical cavity was made in the top of this cake of ice. This cavity was just large enough to allow a daphnid to swim freely; usually the cavity was about 4 mm across and 5 mm deep. When the ice had melted sufficiently almost to fill the cavity an animal was

placed in it by means of a pipette. Great care was taken to introduce no water with the animal. The animals were taken from water at 18° C., placed in the ice water until they ceased to move when touched with a fine needle, and then returned to water at 18°. Two readings were taken with a stop-watch—(1) the time required before the animal became inactive, and (2) the time required by the animal to resume activity when returned to water at room temperature. It is an interesting fact that the animals become inactive by progressive stages, that is, the bodily activities disappear slowly so that the end point is not sharply defined, but when they recover from this "chill-coma" they do so "all at once."

The following data were obtained for *M. macrocopa*. The average time that elapsed before the animals became inactive was three minutes and fifty-three seconds. The actual times varied between five minutes thirty-five seconds and two minutes and forty-two seconds. This period of inactivity (as measured by the time required for recovery after transfer to 18° C.) lasted three seconds. The extremes in this case were nine seconds and two seconds. Fourteen animals were tested.

The data for *P. bidentata* were somewhat different, but not markedly so. The time required to produce "chill-coma" was one minute and thirteen seconds, while the time required for recovery was one minute and forty seconds. The extremes in the first case were one minute fifty seconds and twenty seconds, in the latter case four minutes twenty-five seconds and sixteen seconds.

The other four species reacted similarly. They were observed for an hour and at the end of that time, while showing effects such as reduced heart and gill action, they still responded to touch by vigorous swimming movements. This is in accordance with the fact that all of these species have been found active beneath ice in nature.

Other experiments on subjecting cladocerans to low

temperatures yielded results qualitatively the same. *M. macrocopa,* if continued at 10° for a few hours, soon settles to the bottom and does not swim actively. This ultimately results in death for this species, as the gill chamber becomes clogged with mud and debris from the bottom of the culture bottle. *P. bidentata* will become inactive at a temperature of 2.5° to 3° when the water in which it is swimming is lowered at the rate of 10° per half hour. Other species (*D. magna* and *S. vetulus*) treated in this way will continue active even after the temperature has been lowered and held at 1° C. for an hour.

The species tested fall into two distinct groups, as determined by their ability to withstand sudden immersion in water near zero degrees Centigrade. Two species, *M. macrocopa* and *P. bidentata,* become inactive in from one to four minutes, while the species of *Daphnia* and *Simocephalus* remain active indefinitely at this temperature. It is not at all uncommon for different animals of the same family, genus or even the same species to show differences in response to low temperatures. Blunk (1923) shows that the temperatures at which two species of dytiscid beetle larvae begin feeding are quite different. Mayer (1914) gives data for several marine invertebrates; *Aurelia aurita* from Halifax, Nova Scotia, cease movements at −1.0°, while specimens of the same species collected at Tortugas, Florida, become inactive at a temperature of 7.75° to 11.8° C. Mayer mentions other instances of this same nature. There seems to be no doubt that low temperatures act as barriers to the activity and in many cases to the distribution of animals.

IV

Knowledge on the relation of high temperature to life and activities of organisms can be classified according to the viewpoints of the observers into three groups. Probably the earliest interest in this field came as a result

of observations of the occurrence of animals and plants living in hot springs and other places where the temperature of the water was above that normal for most organisms. The widespread literature on this phase of relationships to supra-normal temperatures has been gathered together by a few writers, such as Wyman (1867), Plateau (1872), Hoppe-Seyler (1875), Davenport and Castle (1896) and Brues (1924). These writers mention the fact that animals and plants have been found living at temperatures between 40° and 70° (sometimes given much higher). There are no records concerning cladocerans in these papers. A second and somewhat overlapping phase of this matter is concerned with the lethal temperatures of animals and plants living within the usual temperature range. Plateau (1872) determined the temperature necessary to kill certain aquatic animals when they were suddenly immersed in hot water. Many other workers were interested in finding upper temperature limits for animal life. Among them may be mentioned Weismann (1876–79), using daphnids; Davenport and Castle (1896), using tadpoles; Vernon (1899), employing many marine animals; Bachmetjew (1899), using insects; and Mayer (1914), working for the most part with coelenterates. Still another series of papers was chiefly concerned with an attempt to explain the nature and course of death brought about by temperatures above normal; here we may mention the work of Davenport and Castle (1896), Dallinger (1887), Loeb (1908, 1916), Demoll and Strohl (1909), Moore (1910), Mayer (1918) and Brown and Crozier (1927–28). Various theories have been advanced as possible explanations of what happens when an animal is killed by heat, and in the last paper just referred to (Brown and Crozier, 1927–28) data derived from experiments on the rate of killing of cladocerans are discussed.

The method employed in the present paper to determine the upper limits of life for various daphnids is a qualita-

tive one, a method used by earlier investigators, and which provides a quick and workable scheme for obtaining a measure of the *relative* differences between species. Such data obtained by one investigator can never be compared directly with those of another, as the details of the method are apt to be different. While one worker immerses the animals quickly in water of the desired temperature, another may warm the water containing the animals gradually, and the rate of warming may vary in different instances. Such unsatisfactory methods of obtaining a true lethal temperature for animals were pointed out by Crozier (1916). He stressed the fact that the *rate* at which an animal is brought to a certain lethal temperature is all-important. And later Jacobs (1919) again emphasized the importance of the time factor and pointed out that different animals differ in their lethal temperatures depending on the time required during heating. The slower rates of increase are more favorable for *Paramecium* and more unfavorable for starfish larvae than the rapid rates, indicating that *Paramecium* can become acclimatized during experiments of short duration. The internal temperature of the animal is also a factor to be considered, particularly if the animal is large or if it moves violently. For these and other reasons, such as the diversity of criteria used to determine death, it is apparent that the only comparisons that can be made are with regard to the relative positions of species on the temperature scale. The true lethal temperatures are determined by locating the point at which the curve for duration of life at lethal temperatures joins the curve of ordinary life-duration (Brown and Crozier, 1927–28).

In the present experiments the animals were transferred with a minimum of water (1 or 2 drops) to a jar containing about 150 cc of water heated to the desired temperature. This temperature could be maintained without fluctuation for several minutes. The animals, one at a time, were placed in water of the desired tem-

perature for one minute and then removed as quickly as possible and examined with a compound microscope. The criterion of death was arbitrarily taken as the absence of motion of the heart. This was usually the last organ to cease movement, although occasionally the eye muscles twitched or the sphincter of the anus contracted after the heart had stopped. The animals never recovered when returned to water at room temperature if "dead" by this criterion. If the temperature to which the animal was subjected was sublethal the animal was discarded and not used for further trials at higher temperatures. In this a consistent, if arbitrary, measure was obtained of the temperature which could be termed a lethal temperature. All the animals used were adult parthenogenetic females; the majority of them were chosen from clones reared for years in the laboratory, but occasionally a few individuals recently collected were used to test for possible differences due to seasonal rhythm. No such differences were discovered.

The species used in these experiments, together with their lethal temperatures, are given in Table I. The temperatures are rounded off to the nearest whole degree, as the observations probably are exact to only half a degree. In the third column are some results for a few of the species as given by Weismann (1876–79). And in the last column are given the probable geographical and seasonal distributions of the species.

It will be seen from the table that there is a distinct relation between the prevailing temperature for the occurrence of a cladoceran species and its lethal temperature. The values of the lethal temperatures as determined by Weismann are from three to six degrees lower than the temperatures fixed by the writer, but they differ less than two degrees from other experiments in which the water containing the daphnids was heated gradually. The data from Weismann were obtained by heating the water gradually (three to six hours). These observations which tend to show that cladocerans when subjected to a

TABLE I

THE TABLE SHOWS THE LETHAL TEMPERATURES FOR SEVERAL SPECIES OF CLADOCERANS, AS DETERMINED BY SUDDENLY IMMERSING THE ANIMALS IN WATER OF THE DESIRED TEMPERATURE FOR ONE MINUTE. A FEW VALUES DETERMINED BY SLOWLY INCREASING THE TEMPERATURE TO A LETHAL POINT ARE GIVEN FOR SOME OF THE SPECIES (FROM WEISMANN, 1876–79). IN THE LAST COLUMN THE KNOWN GEOGRAPHICAL RANGE AND SEASONAL RHYTHM ARE GIVEN.

Species	Lethal temperature	Lethal temperature Weismann	Distribution
Macrothrix rosea	50		Probably southern
Moina macrocopa	48		Wide-spread; summer maximum
Pseudosida bidentata	48		Southern
Moina rectirostris	47	42	Wide-spread; summer maximum
Latanopsis occidentalis	46		Probably southern
Daphnia pulex (typical)	44	38	Wide-spread; spring and fall maxima
Simocephalus vetulus	43	38	Wide-spread; spring and fall maxima
Simocephalus exspinosus	43		Wide-spread; spring and fall maxima
Scapholeberis mucronata	43		Wide-spread; (rhythm undetermined)
Ceriodaphnia laticaudata	43		Wide-spread; spring and fall maxima
Daphnia longispina	42	38	Wide-spread; spring and fall and sometimes winter maxima
Daphnia magna	41	38	Northern
Daphnia pulex (984)	41		Wide-spread; maximum earlier in spring than typical D. pulex
Sida crystallina	40	35	Probably northern; spring? and fall maxima

slowly rising temperature die at a lower temperature than when suddenly immersed, would, according to Jacobs (1919), indicate that they show little or no acclimatization to temperature during the interval of the experiment. The lethal temperatures of the different species, by whatever method determined, have the same positions relative to one another. Observations by Mayer (1914) on comparisons of the lethal temperatures for marine inverte-

brates from different geographical regions of the Atlantic Coast and the rather fragmentary work of Huntsman and Sparks (1924) provide further evidence of the interdependence of the distribution and lethal temperatures of animals.

SUMMARY

(1) Cladoceran species may be listed as southern, wide-spread or northern. Those that are wide-spread and northern have definite seasonal rhythms, some species being abundant in the summer and others in the spring and fall.

(2) The summer and southern forms, as many as were tested, become inactivated quickly by low temperatures, while the northern forms or the species having spring and fall maxima remain active indefinitely at the same low temperature.

(3) When the fifteen clones and species were arranged according to the magnitude of their lethal temperatures, it was found that they formed a good series, the southern species and those with summer maxima having the highest lethal temperatures, the forms having spring and fall maxima next, and the northern species having the lowest lethal temperatures.

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RAYMOND PEARL ON THE DOCTRINE OF "LIKE PRODUCES LIKE"

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PEARL's recent attack on eugenics in the November, 1927, number of the *American Mercury* (presumably a suitable medium for disseminating biological views!) is important because his scientific reputation may lead many persons to accept his conclusions without realizing the inadequacy of his interpretations.[1] Of even more significance is the fact that his original paper, presented before the Fifth International Congress on Genetics held in Berlin in September, 1927, has been reprinted in pamphlet form for distribution primarily among American students of sociology.[2] These two publications are essentially the same in content and point of view.

Granting that mankind always will have need for superior men as its leaders, Pearl objects to the usual eugenical program for producing such men. He insists that the science of genetics does not unreservedly support the doctrine that superior men are to be produced by persuading superior people to breed more and inferior people to breed less. For this reason, he proceeds to investigate the question *de novo*, putting aside the earlier investigations of Galton and Ellis. Incidentally his reinvestigation utilizes a method similar to that of Galton and Ellis. Pearl culls from biographies of 1,011 eminent persons in the Encyclopaedia Britannica the data on the intellectual status of their fathers. He wisely restricts this inquiry to 588 persons "whose distinction is in the main derived solely from their own personal superiority."

[1] R. Pearl. "The Biology of Superiority," *American Mercury*, November, 1927, 257–266.

[2] R. Pearl. "The Present Status of Eugenics." Sociological Press, Hanover, New Hampshire. 1928. pp. 1–20.

Of these 588 men of "pure" eminence, sixty-three were philosophers, eighty-five were poets and sixty-six were scientists. Data relating to the parentage of these three groups are then presented. He desires to determine whether or not the fathers of these eminent philosophers, poets and scientists were as distinguished as were their sons. His test of eminence, as for the sons, is the amount of space devoted to them. Of the sixty-three philosophers, only two fathers and one mother were sufficiently distinguished to leave public record of that fact in the pages of the Encyclopaedia Britannica. Similar results were found for the poets and scientists.

Pearl does not argue, however, that such a criterion is completely adequate to determine the intellectual status of the parents of these eminent men, as shown by the fact that he proceeds to list in detail the occupations held by these parents. For example, the occupations of the fathers of the philosophers are reported as follows: Six petty political office holders, five higher political office holders, four merchants and shopkeepers, four lawyers, four clergymen of small parishes, four college or university professors, three physicians, two watchmakers, two weavers, two farmers or peasants, two of titled family, one each of soldier, "citizen of London," "saddler," "illiterate and criminal," manufacturer, clerk, shoemaker, fisherman, historian and schoolmaster. He then attempts to interpret the significance of these occupations. It is precisely at this point that Pearl is most vulnerable. Quoting:

> Taking the list of fathers as a whole it is perhaps *as fair a cross-section of men in general* as one could expect to attain in a sample of forty-eight. *It is mainly composed of mediocre people* . . . as a matter of fact, the particular combinations of genes which made these greatest philosophers were derived from about an *average lot of human beings.* (Italics not in original.)

He is so convinced that these eminent men have issued from mediocrity that he designates any attempt to interpret the data otherwise as "arrant nonsense."

Similar data and the same interpretation are given for the fathers of poets and scientists.

Do the occupations listed really indicate that the fathers of these distinguished philosophers, poets and scientists represent a mere cross-section of men in general? Fortunately, the reader need not accept Pearl's unsupported opinion on this crucial question, since sufficient work now has been done to warrant the use of an objective standard of reference whereby the intellectual level of an important occupation may be determined. We refer here to the established fact of an intellectual hierarchy of occupations, first suggested by Taussig,[3] later verified by the army intelligence tests[4] and extensively elaborated by Barr using subjective ratings[5] and by Fryer using intelligence tests in a civilian employment and vocational bureau.[6]

Let us apply the Barr scale to Pearl's data and compare the results with a sample of the general population as given by Terman.[7] The results of this procedure are presented in Table I. Where there is a question as to including a vocation in two possible categories it is always included in the lower classification. Barr ratings could be applied to the occupation of the fathers of thirty-three philosophers, forty-five poets and thirty-seven scientists. Incompleteness of ratings is due to incompleteness of Pearl's data (no occupation being given for fathers of fifteen philosophers, thirteen poets and fifteen scientists) and to inadequate occupational description. As a matter of fact, for most of the "unclassifiable" occupations, had we been less conservative, we

[3] F. W. Taussig. "Principles of Economics, II." New York, Macmillan. 1912. pp. 134–138.

[4] "Psychological Examining in the United States Army," Memoirs of the National Academy of Science, 1921, XV, Part III, Ch. 15, "Intelligence Ratings of Occupational Groups," 819–837.

[5] L. M. Terman, "Genetic Studies of Genius, I." Stanford University Press, 1925. pp. 66–72.

[6] D. Fryer. "Occupational Intelligence Standards," *School and Society*, 16: 273. 1922.

[7] *Op. cit.*, p. 72.

TABLE I

COMPARISON OF OCCUPATIONS OF FATHERS OF EMINENT PHILOSOPHERS, POETS AND SCIENTISTS, CLASSIFIED BY THE BARR SCALE, WITH ADULT MALES OF THE GENERAL POPULATION

Barr Rating	Fathers of Philosophers		Fathers of Poets		Fathers of Scientists		Adults of the General Population 1910 U. S. Census, California
	Number	Per cent.	Number	Per cent.	Number	Per cent.	Per cent.
15 or above ...	21	63.6	15	33.3	18	48.6	2.2
12–14	2	6.1	4	8.9	5	13.6	4.5
9–11	5	15.1	17	37.8	3	8.1	37.0
6–8	2	6.1	—	—	2	5.4	13.4
3–5	3	9.1	9	20.0	9	24.3	42.9
0–2	—	—	—	—	—	—	—
Total	33	100.0	45	100.0	37	100.0	100.0
Unclassifiable	15		27		14		
No record	15		13		15		
Grand Total	63		85		66		
Average rating	13.27		11.07		11.69		7.92
Standard Deviation ...	± 4.14		± 4.38		± 5.01		± 3.38

could have rated them as being sufficiently similar to other occupations to warrant a rating. Had this been done, there is evidence that these additional "doubtful" ratings would have been relatively high, falling for the most part in the upper levels. This may be seen from the following "unclassifiable" occupations in the case of fathers of philosophers, poets and scientists: Eleven petty political office holders, twenty-five of titled family or wealthy, five weavers, one tanner, three army officers, three poets, two soldiers, two watchmakers, one priest of idol, one "citizen of London," one "illiterate and criminal" and one libertine.

A mere glance at Table I shows the intellectual superiority of the fathers of these eminent men as contrasted with standards for the general population. Roughly 69

per cent. of the fathers of philosophers, 42 per cent. of the fathers of poets and 62 per cent. of the fathers of scientists receive Barr ratings of 12 or more, indicating marked intellectual superiority. In contrast, we note that only 7 per cent. of Terman's sample of the general population rate as high. Here is convincing evidence that the fathers of these eminent men are themselves superior. By no reasonable interpretation can they be dubbed "average," "mediocre" or "a mere cross-section."

This same trend is found regardless of the particular method we use to classify the occupations of fathers. When Taussig's scale is applied to Pearl's data the results, as shown in Table II, reveal a negligible contribution from the two lowest social-economic classes and a major contribution from the upper two classes. Indeed, about 70 per cent. of these fathers come from the upper two classes in society. In other words, Pearl's data when interpreted by standardized rather than by personal methods show just the opposite of his own contention. *The fathers of these eminent men are as a group superior in ability.*

It may be well to present additional results tending to show that Pearl's data are entirely in harmony with other research studies in the field of genius. Catherine Cox in her recent elaborate study of genius presents a table showing the Taussig classification of the occupation of the fathers and maternal grandfathers of 282 of the 300 most eminent men in history (confined, as in Pearl's study, only to those achieving eminence on the basis of personal merit).[8] This table is reproduced as Table III. One reason for presenting Cox's data at this point is to demonstrate with far more complete data that Pearl's results are truly typical. Again, the evidence points unmistakably to the fact that eminent persons, *for the most part,* spring from superior intellectual human stock.

[8] C. Cox, "Genetic Studies of Genius, II." Stanford University Press, 1926. p. 37.

TABLE II

CLASSIFICATION OF OCCUPATIONS OF FATHERS OF EMINENT PHILOSOPHERS, POETS AND SCIENTISTS BY THE TAUSSIG SCALE (PEARL'S DATA)

Taussig Scale	Fathers of Philosophers		Fathers of Poets		Fathers of Scientists		All Combined	
	Number	Per cent.	Number	Per cent.	Number	Per cent.	Number	Per cent.
Professional	22	50.0	19	36.5	24	53.3	65	46.1
Semi-professional	11	25.0	20	38.5	4	8.9	35	24.8
Skilled	8	18.2	11	21.2	16	35.6	35	24.8
Semi-skilled	3	6.8	2	3.8	1	2.2	6	4.3
Common labor	—	—	—	—	—	—	—	—
Total	**44**	**100.0**	**52**	**100.0**	**45**	**100.0**	**141**	**100.0**
Unclassifiable	4		20		6		30	
No record	15		13		15		43	
Grand total	**63**		**85**		**66**		**214**	

TABLE III

OCCUPATIONAL STATUS OF THE FATHERS AND MATERNAL GRANDFATHERS OF 282 EMINENT MEN (AFTER COX)

Taussig Classification	Fathers		Maternal Grandfathers	
	Number	Per cent.	Number	Per cent.
I Professional	148	52.9	77	41.8
II Semi-prof; Higher business; Gentry	81	28.9	65	35.3
III Skilled workmen and Lower business	37	13.2	35	19.1
IV Semi-skilled	11	3.9	3	1.6
V Unskilled	3	1.1	4	2.2
Total	280	100.0	184	100.0
No record	2		98	
Grand total	282		282	

It is to be noted that this same tendency may be traced back even to the maternal grandfathers. Genius does not come primarily from the "mediocre" element in society as Pearl would have us believe.

These findings of Cox verify previous studies of this same subject. Galton found that the overwhelming majority of the ninety-six contemporary leading men of science in Great Britain came from the upper classes and only two from the artisan or peasant class.[9] Ellis found that of 829 British men of genius, 18.5 per cent. came from the upper classes (or "good family"), 41.3 per cent. from the professions, 31.2 per cent. from the manufacturing and commercial classes and only 2.5 per cent. from the artisan and laboring classes.[10] Cattell's figures for 885 American men of science show substantially the same trend, 43 per cent. of our leading men of science having come from the professional classes.[11] Space for-

[9] F. Galton. "English Men of Science." New York. Appleton. 1875. pp. 16–18.

[10] H. Ellis, "A Study of British Genius." London. Hurst and Blackett, Ltd. 1904. pp. 77–78.

[11] J. McK. Cattell, "Families of American Men of Science," *Popular Sci. Mo.*, May, 1915, 86: 504–515.

bids mention of important studies conducted by De Candolle, Odin, E. L. Clarke and others, all of which point in the same direction.

Finally, a definite relationship between the tested intellectual ability of the most gifted children in California and the intellectual level of their fathers' occupation has been conclusively established by Terman's recent study.[12] The results are summarized in Table IV. Again the predominating contribution of the upper social-economic

TABLE IV

COMPARISON ON TAUSSIG SCALE OF THE OCCUPATION OF THE FATHERS OF PHILOSOPHERS, POETS AND SCIENTISTS (ALL COMBINED) WITH THE FATHERS OF TERMAN'S GIFTED CHILDREN

Taussig Classification	Fathers of Philosophers, Poets and Scientists (all combined)		Fathers of Terman's Gifted Children	
	Number	Per cent.	Number	Per cent.
Professional	65	46.1	176	31.4
Semi-professional	35	24.8	280	50.0
Skilled	35	24.8	66	11.8
Semi-skilled	6	4.3	37	6.6
Common labor	.....	.....	1	0.13
Total	141	100.0	560	99.93
Unclassifiable	30		.....	
No record	43		.....	
Grand total	214		560	

levels is apparent. Furthermore, the percentages for Terman's group approximate Pearl's group, although the members of the former are now living in California and the members of the latter long since have been dead.

The writers have no desire to discuss detailed aspects of Pearl's papers or to argue at length regarding "propaganda," or that outlandish eugenic programs exist which propose to sterilize the lower 95 per cent. of

[12] *Op. cit.*, p. 64.

the population (implied by Pearl). We do contend, however, that Pearl has succeeded in giving a most misleading impression when he argues that the "eminent" spring from the "mediocre." We find nothing in Pearl's data in conflict with those of Galton, Ellis, Cattell, Cox or Terman. Indeed, all this evidence reinforces the desire of the eugenist to bring about an increasing marriage-rate and an increasing birth-rate in the upper levels of society. If all this be "arrant nonsense," then quantitative fact succumbs again to *obiter dicta* from the scientist's armchair.

SHORTER ARTICLES AND DISCUSSION

FISHER'S THEORY OF DOMINANCE

THE phenomenon of dominance in Mendelian heredity has from the first presented certain puzzling features. There was at one time a tendency to look upon dominance as a principle of heredity on a par with the principles of gene autonomy, the orderly segregation of allelomorphs and the assortment of non-allelomorphs. It was soon discovered that dominance is often merely a superficial appearance which disappears on careful examination and that even superficial dominance is far from invariable. It became clear that in any case dominance is a phenomenon of the physiology of development to be associated with the various types of epistatic relationships among factors rather than with the more fundamental genetic principles cited above.

The reasons for the undoubted frequency of real dominance, and for the also undoubted tendency of the type to be dominant over the mutant, are not obvious. A possible explanation has been developed by R. A. Fisher in two recent papers (2, 3) according to which these tendencies are statistical consequences of natural selection rather than of anything inherent in the physiology of gene action. Assuming that the pristine character of heterozygotes is intermediate, his suggestion is, in brief, that the observed gene mutations, however rare in laboratory experience, have nevertheless had opportunity to recur a very large number of times in the evolutionary history of the species; that such mutations are usually deleterious; that natural selection plays with much greater force on the heterozygotes than on the homozygous mutants (because of the enormously greater relative abundance of the former); that the character of the heterozygote is subject to the action of modifying factors, and that in consequence of all these facts there will be a gradual drift of the heterozygote toward the wild type, not necessarily involving the homozygous mutant type to any great extent. In the course of geologic time this may bring about complete dominance of the wild type or dominance so nearly complete that the heterozygote is no longer subject to adverse selection. He concludes that "with mutation rates of the order of one in a million, the corresponding selection in a state of nature, though extremely slow, can not be safely neglected in the case of heterozygotes."

This suggestion merits careful consideration, not only because of its interest on the evolutionary side, but especially because of its bearing on any deduction as to the nature of gene action which might be based on dominance. Fisher does not present his calculation of the selection rate due to this cause. The present writer reaches a figure so "extremely slow" as to make its efficacy seem highly questionable.

Suppose that a large population is in approximate equilibrium between the pressure of recurrent mutation from type factor A to a and adverse selection of heterozygotes as well as of homozygous mutants. It will be assumed that these are autosomal genes, as more favorable to the theory than sex-linked ones. Let q be the proportion of A's and $(1-q)$ that of a's in the initial constitution of a generation and assume that individuals of the three phases AA, Aa and aa are successful in attaining parenthood in the proportions $1:1-hs:1-s$, respectively. The initial zygotic proportions $(1-q)^2\,aa+2q\,(1-q)\,Aa+q^2\,AA$ become at the time of gametogenesis:

$$\frac{(1-s)\;(1-q)^2\,aa+2\;(1-hs)\;q\;(1-q)\;Aa+q^2\,AA}{1-2hsq\;(1-q)-s\;(1-q)^2}$$

The proportion of A's increases slightly:

$$q_1=q\left[\frac{1-hs\;(1-q)}{1-2hsq\;(1-q)-s\;(1-q)^2}\right]$$

The initial zygotic constitution of the next generation would show this increase were it not for a tendency for A to mutate to a, which at equilibrium just balances it. Let u be this mutation rate per gamete. Then $q_1-q=uq_1$ giving $q^2(1-2h)-q(2-3h+hu)+1-h-\frac{u}{s}+hu=0.$

For values of h larger than $\sqrt{\frac{u}{s}}$ this yields for the equilibrium value of q approximately $q=1-\frac{u}{hs}$.

Since $\sqrt{\frac{u}{s}}$ is in general a small fraction, this formula holds until selection against the heterozygote has almost ceased. The value for complete dominance (at least with respect to selection effects) is $q=1-\sqrt{\frac{u}{s}}$, and is approached only as h becomes

smaller than $\sqrt{\frac{u}{s}}$. These values agree approximately with those reached by Fisher for the equilibrium conditions.

Suppose that there is another factor, M, which modifies the selection against the heterozygote, giving it a reproductive rate with the ratio $1-h's$ to that of the wild type instead of $1-hs$. The reproductive ratios for mm and M– will be as follows:

$$\begin{array}{lll} & \underline{AA} & \underline{Aa} \qquad\qquad\qquad aa \\ mm & q^2+2(1-hs)\,q(1-q)+(1-s)(1-q)^2=1-2u \text{ approximately} \\ M- & q^2+2(1-h's)\,q(1-q)+(1-s)(1-q)^2=1-2\frac{h'}{h}u \text{ approximately} \end{array}$$

Thus the selection against *Aa* (and *aa*), however intense, will carry with it a selection in favor of M– as compared with mm of only about $(1+2u(1-\frac{h'}{h}):1)$.

The advantage of the modifier is thus decidedly small. Even if it is able at once to bring the heterozygote *Aa* to equality with the wild type in reproductive rate ($h'=0$) the pressure per generation toward its fixation (which is $2up^2(1-p)$, where p is the proportion of m's) is never more than half that which would result from recurrent mutation from m to M at the same rate as that from A to a (*i.e.*, up). It may be doubted whether mutation pressure by itself has been important in fixing factors.

It might be suggested that mutation pressure is the factor which reduces useless vestigial organs beyond the stage at which they can exert any conceivable deleterious effect, *e.g.*, in the frequently cited case of the whale's hind legs; and that another evolutionary force of the same order of magnitude can not be considered negligible. It is common experience that mutations tend to reduce development of parts. It is only necessary to compare the number of mutations which reduce wing size or venation, size of eye or eye pigmentation, number and size of bristles, etc., in Drosophila as compared with those with the opposite effects to find an illustration of this principle. The explanation usually advanced, that random changes in a gene are more likely to lead to injury to developmental processes than stimulation, seems a reasonable one. Mutation pressure would thus, no doubt, tend to reduce the development of useless characteristics. It is probable, however, that there is a more important factor in this case. It has been shown that genes often have multiple effects and it is not unlikely in view of results such as those of Dobzhansky (1)

that in general any given gene has some effect on nearly all parts of the organism. Thus the evolutionary changes in a system of genes, some bringing increased development and others reduced development to those parts of the organism which are under direct selection, should have a net effect on *indifferent* parts in the direction of reduced development. The reduction of indifferent vestigial organs need not therefore be looked upon as the direct consequence of mutation pressure but as a by-product of multiple selection pressures of a higher order of magnitude. In the case of modifiers of dominance there seems to be no reason why increase rather than decrease of resemblance to wild type should come about as a by-product of the general selection process.

It will be seen that the hypothesis that a selection pressure, of the order calculated here, can be the *general* factor making for dominance of wild type, depends on the assumption that modifiers of dominance (assumed to be sufficiently abundant) are in general so nearly indifferent to selection on their own account that a force of the order of mutation pressure is the *major* factor controlling their fate. With the prevalence of multiple effects in mind it seems doubtful to the present writer whether there are many such genes.

It should be emphasized, however, that this conclusion rests to a large extent on the low frequency of even heterozygous mutants, to be expected where mutation is balanced by adverse selection of a higher order. If for any reason the proportion of heterozygous mutants reaches the same order as that of the type, selection of modifiers of dominance approaches the order of direct selection in its effects and might well become of evolutionary importance. Fisher's argument for the importance of this factor under certain conditions of artificial selection seems valid and must be taken into account in interpretation of dominance in domestic animals, especially, perhaps, in poultry. It seems unlikely that similar conditions would occur in nature except in special cases.

If this interpretation of the dominance of wild type allelomorphs is not available, what alternative is there? Probably most geneticists would hold that dominance in general has some immediate physiological explanation. Bateson long ago suggested that pairs of allelomorphs represent the presence and absence of something and that it was to be expected that one dose of an entity would give a result more like that of two doses than

like complete absence. There are many reasons which have led to the general abandonment of the presence and absence hypothesis in its literal form. There is still much to be said, however, for the idea that the commonest type of change in a gene is one which partially or completely inactivates it in one or more respects. The effects of mutant genes, and especially of intermediate mutant allelomorphs, in combination with known deficiencies indicate differences from wild type of a negative rather than a positive sort.

On the view that genes act as catalysts and largely through bringing about the production of catalysts of a second order, it is easy to show that increase in the activity of a gene should soon lead to a condition in which even doubling of its immediate effect brings about little or no increase in the ultimate effects. Under such conditions, there would be dominance of the active phase. Lower levels of activity of the gene would show more or less imperfect dominance, corresponding to the usual case among intermediate allelomorphs in multiple series. Increased activity of other genes affecting transformation of the same substrate would also reduce dominance. Conversely, reduction of activity of such modifiers gives increase in dominance and thus the kind of effect required in Fisher's theory. There seem to be only rather special conditions (*e.g.*, certain threshold effects) under which it is to be expected that heterozygotes will be closer in effect to the inactivated than the active gene, making the latter recessive. Without going into further detail, it seems that in the hypotheses that mutations are most frequently in the direction of inactivation and that for physiological reasons inactivation should generally behave as recessive, at least among factors with major effects, may be found the explanation of the prevalence of recessiveness among observed mutations.

There remains the difficulty emphasized by Fisher that whatever the status of the genes in a species at a given time evolution through replacement of type genes by mutant allelomorphs, in general more or less completely recessive, would ultimately bring it to a condition in which the type genes would no longer be prevailingly dominant, were there no process continually at work increasing the dominance of new type genes. This argument, however, seems to involve the assumption that the array of observed gene mutations is a fair sample of those which are seized upon and fixed in the evolutionary process. This can hardly be the case if most such mutations are of the nature of inactivation.

Probably few geneticists, including Bateson himself, have ever taken very seriously the latter's suggestion that evolution may have taken place wholly by losses from a primitive complex. Presumably a species remains about the same for long periods of geologic time in the average degree of physiological activity of its genes. The mutations which actually are *fixed* must thus as frequently involve increases in activity as decreases. Under such an evolution, the species may continue indefinitely in a condition in which the most frequently *occurring* major mutations are recessive.

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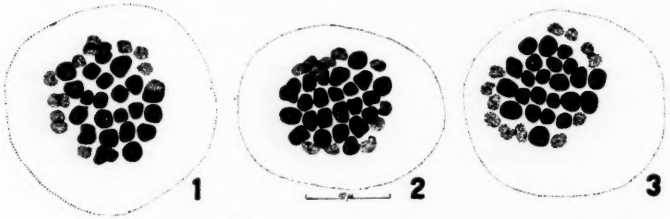
INTERSPECIFIC HYBRIDIZATION IN NICOTIANA. X. HAPLOID AND DIPLOID MEROGONY

NICOTIANA DIGLUTA, a synthetic amphidiploid species with 36_{II} chromosomes (24_{II} of *N. tabacum* + 12_{II} of *N. glutinosa*, Clausen and Goodspeed, 1925), exhibits a conspicuously higher frequency of distributional irregularities than most normal species. In order to obtain a quantitative measure of this phenomenon, *digluta* (carmine) ♀ was crossed with white *tabacum* ♂ under the assumption that any F_1 plants resulting from *digluta* gametes lacking the *W*-bearing *tabacum* chromosome would be *pink* rather than *carmine* in color. The experiment failed of its purpose, inasmuch as only one pink plant was obtained among 173 F_1 plants grown, and it had $23_{II} + 11_{II}$ chromosomes (Figure 2) instead of $23_{II} + 13_{I}$, as was to be expected. However, cytological examination of other plants in the progeny also showed that chromosomal elimination was occurring in *digluta*. Figures 1 and 3 are from plants having $22_{II} + 13_{I}$ and $23_{II} + 12_{I}$, instead of the normal number $24_{II} + 12_{I}$ expected in F_1 *digluta-tabacum*. These figures are presented mainly for comparison with those which follow.



FIGURES 1–3. Silhouette drawings of typical PMC of three different F_1 *digluta-tabacum* plants from the same population as that in which a haploid *tabacum* appeared. Figure 1, $22_{II}+13_I$; 2, $23_{II}+11_I$; 3, $23_{II}+12_I$; univalent chromosomes stippled.

Among the 173 F_1 *digluta-tabacum* plants one, however, was strikingly exceptional in having small white flowers and other morphological features distinctly different from those of normal F_1 plants. Cytological examination of this plant, 28234 P26, clearly revealed its chromosome number as 24_I (Figures 4 to 6), indicating it to be a haploid *tabacum*. As with other *tabacum* haploids excellent cytological preparations were readily obtained by making smears of PMC in aceto-carmine. Cells like that illustrated in Figure 4, showing 24 widely scattered chromosomes, were not uncommon; and even cells of the type shown in Figure 5, illustrating random distribution of univalents, were easily countable despite the crowding of the chromosomes. Less commonly cells were observed in which the chromosomes had divided (Figure 6). In this figure, four undivided univalents are clearly seen at one pole, one at the other, and the remaining 19 univalents have divided, but apparently one half-univalent is strung out in the middle region and has broken into two parts. The figure is interesting because it indicates that non-reduction, presumably leading to dyad production, may not be a completely equational process. Examination of later stages revealed the presence of a considerable percentage of dyads and of other irregularities in pollen production which have been shown to be characteristic of haploid *tabacum*. The cytology of this plant was in every respect identical with that of other haploid *tabacum* plants which we have under observation.

The morphological features of the plant were those characteristic of haploid *tabacum*. The paternal parent was identical morphologically with *N. tabacum* var. *purpurea*, except that it had white instead of carmine flowers. The haploid here described

FIGURES 4–6. Silhouette drawings of PMC of 28234 P26, a haploid *tabacum* plant appearing in an F_1 population of *N. digluta* ♀ × *N. tabacum* ♂. Figure 4, 24_I chromosomes widely scattered in the cell; 5, 24_I chromosomes undergoing random distribution; 6, one undivided univalent at the left pole, four at the right, the remaining 19 divided with one half-univalent strung out in the mid-region. Magnification the same as in Figures 1–3.

was identical with *purpurea* haploids, of which a number have been obtained, except that it had white instead of carmine flowers. It was completely sterile, as are the *purpurea* haploids. There seems to be no escape from the conclusion that it represents a true case of haploid merogony.

The discovery of this plant recalls the circumstances attending the origin of *digluta* (*cf.* Goodspeed and Clausen, 1925). In the population in which *digluta* was found, arising from *glutinosa* ♀ × *tabacum* var. *purpurea* ♂, to quote, "The third plant was very strikingly different from the other two; in fact, if our notes and memory may be depended upon, it was identical with the *purpurea* haploid which was obtained later." Unfortunately this plant was not examined cytologically, but it was probably a haploid *tabacum* arising from a male nucleus as in the instance described above.

The demonstration that haploid merogony may occur in flowering plants suggests that certain phenomena of interspecific hybridization may be due to diploid merogony. Thus in backcrosses of F_1 *sylvestris-tabacum* ($12_{II} + 12_I$) to *sylvestris* (12_{II}) occasional plants are produced which have 12_{II} chromosomes and which are identical in every respect with *sylvestris*. A case in point has been described by Goodspeed and Clausen (1927). Among 21 plants from F_1 *sylvestris-tabacum* ♀ × *sylvestris* ♂, one was observed to be identical with *sylvestris*. It was the only plant having 12_{II} chromosomes, and a thorough examination of it revealed no differences whatever from *sylvestris* either in

cytology or morphology. Pollen condition and fertility were fully equivalent to that exhibited by normal *sylvestris*. Two selfed progenies have been grown from it: 26153 consisting of 49 plants, all normal *sylvestris* except one which was a spontaneous trisomic; and 28398 also consisting of 49 plants, all of the normal *sylvestris* type. Assuming random distribution of univalents and independent assortment of bivalents in F_1 *sylvestris-tabacum*, the chance of obtaining a 12-chromosome gamete is very small, only one in 4,096, and the chance in turn that such a gamete will contain nothing but *sylvestris* chromosomes is again only one in 4,096. It is suggested, therefore, that the occasional pure *sylvestris* plants obtained from F_1 *sylvestris-tabacum* ♀ × *sylvestris* ♂ probably owe their origin to diploid merogony.

In both the above instances the question of a possible influence of maternal cytoplasm arises. In the haploid *tabacum* described above, the maternal cytoplasm is from *N. digluta* and traces back through it to *N. glutinosa*. The maternal cytoplasm of the *sylvestris* plants traces back through F_1 *sylvestris-tabacum* to *N. tabacum*. In neither case, however, was any influence of the maternal cytoplasm discernible.

SUMMARY

(1) In an F_1 population of *N. digluta* ♀ × *N. tabacum* ♂ a single haploid *tabacum* plant was secured in a population of 173 individuals.

(2) It is suggested that the occasional pure *sylvestris* plants obtained in backcrosses of F_1 *sylvestris-tabacum* ♀ × *sylvestris* ♂ may arise from diploid merogony.

(3) No detectable influence of maternal cytoplasm was discernible in the above described instances.

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THE CLEANING HABITS OF A KATYDID

DURING the winter of 1927–28 I had under observation a meadow katydid *Orchelimum agile* De Geer which I captured just before winter came on. This katydid was kept in a quart glass jar covered with a piece of thin muslin. It was given daily fresh pieces of lettuce leaf. Now and then orange juice was offered it by moistening the end of a broom straw with it, and tiny grains of moist sugar were given it in the same manner. The little insect seemed quite at home, and became a pet with all the household. Its dainty cleaning habits soon excited the attention and admiration of all, and many interesting notes on this behavior were obtained. All sentient creatures have a highly developed sense of cleanliness. In some instances the deposition of the excreta is a matter of considerable attention, as in the case of the domestic cat and of many birds caring for their helpless nestlings. In addition to this a nice sense of bodily cleanliness is observed, and the lowliest insects may have this sense in an eminent degree. The little katydid in question proved so interesting in this respect and showed such niceties of judgment in the matter that I shall give the details of the various operations.

The ordinary grasshopper or katydid is veritably a nearly rigid box of chitin, with three legs on each side and a pair of wing-covers. Beneath the external wing-covers or tegmina are the true membranous flying wings. The antennae or feelers consist of two fine capillary, many-jointed, sensitive structures which are attached between the eyes, and constantly play like whips around the insect. In the case of the katydids they greatly exceed the entire length of the body. Men probably do not know all the functions of these sensitive antennae, but we know enough to feel certain that they enable the insect to adjust itself with much nicety to its immediate surroundings. To be sure the moon is not within a katydid's reach, but what does a katydid care about lunar distances or stellar magnitudes? He worries alone about the details of his immediate world, which he can step on or reach with foot or antenna. Unquestionably they play a part in enabling him to judge distances, and he well knows that anything within reach of his antennae is not far away from his feet.

These delicate, restless antennae come in for much careful cleaning. Now and then he pulls one down with his front foot so that he can bring it to his mouth parts. It is then run very

slowly through his mouth parts from the base to the apex. As it is moved toward the apex, it bows out in an ever-enlarging loop until the tip is reached and released. The other antenna is now washed in the same manner. In pulling the antennae down to the mouth, the left front leg is used for the left antenna, and the right is used for its corresponding antenna. Since the antennae are inserted near the top of the head at a considerable distance above the mouth parts, it is evident that a goodly length of the basal portion can not be drawn through the mouth. This does not worry the little katydid in the least. It now moistens the tarsal joints of the front leg beneath and draws the foot again and again over the unwashed portion, beginning well back of the eyes and coming down upon the antennae, exactly as a cat washes its face. From time to time the moistening is repeated, for the little insect is not satisfied with a mere superficial dry brushing. This dispenses with the antennae. The face and eyes are now washed. This is necessarily done only with the front legs, for they alone can reach the upper portions of the face. The movements are precisely like those of a cat or a fly doing the same thing. First the tarsal joints beneath are moistened, then the foot is brought far up back of the eyes, and swept downward to the mouth with a smooth, even draw several times repeated. The tarsal joints are again moistened and the movements repeated. There are times when it is obvious the insect is making every effort to reach as far back upon the top of its head as it is anatomically possible with its fore feet. Each front leg must be used for its own corresponding side of the head, for the feet or hands as one chooses to call them can reach but little beyond the frontal area toward the other side of the head. With these nicely adjusted washing movements the eyes can be cleaned, and even the sides of the head and face by careful manipulation of the angle of the femur and tibia.

It was a long time before I saw the middle pair of legs used in the cleaning operations, and I had concluded that they could play very little part in such matters. I found I was much mistaken. Nature suddenly refuted my dogmatism with one of the most beautiful illustrations of the part they, too, play in this matter of body-hygiene.

One day I saw the little katydid bring its hind leg and thigh far out one side until the thigh was at about right angles with

the body. It was a very stiff and awkward position and apparently not a comfortable one for the insect. It had something in mind, however, so I watched intently its every movement. It now proceeded to moisten the tarsal under surfaces of its middle leg on that side. This done, it very deftly brought the foot up and far back upon the long, fat thigh of the extended hind leg, and drew it downwards smoothly toward the tibia. Alternate moistening and stroking movements were usually the rule. The feet of this middle leg were brought back well toward the body and drawn over a good portion of the outer area of the hind thigh by careful adjustments of the movements. It was a marvelous instance of a studied adaptation to the difficulties of the situation, yet it was all done with a nicety of judgment and precision that called for the highest admiration. Now we shall see how the hind legs are used in this problem of body-hygiene. Ah, here comes another masterly handling of the various anatomical features, excelling even the nicely adjusted movements of the middle legs used in cleaning the thighs of the rather inaccessible hind legs.

While watching the katydid engaged in its usual leisurely singing, I saw it stretch its hind legs well out behind it. The body was raised enough to allow one hind leg, with the tibia closed snugly against the femur, to be thrust beneath it until the foot was within reach of the mouth parts. The under surface of the tarsal joints was then moistened. The leg was now extended backwards, and moved around in a series of groping kicks until the opposite hind leg was found. When it had located it with its foot, it began deliberate rubbing movements over the inner side and top of the thigh, drawing the foot along smoothly toward the tibia each time, but not over the tibia. The moistening act was repeated, followed by additional cleaning strokes of the hind femur, in spite of the somewhat difficult movements involved in bringing the hindermost foot to the mouth each time. At another time the tarsal joints of the hind leg were made to stroke the upper surface of the wing-covers, beginning at a point near the middle and pushing the feet along toward their tips. On several occasions the thigh of the hind leg was hugged close to the body and rubbed vigorously, as if to scratch the sides of the body, but whether this was intended to clean the inner side of the thigh or the side of the body or whether only

to allay irritation is not entirely plain. The tarsal joints of the hind tibia are sometimes used to rub along the sides of the abdomen as far as the anal structures.

The posterior portion of the abdomen with the cerci and related structures comes in for an intensive overhauling now and then. It is no easy matter to bring the tip of the abdomen to the mouth parts, but by sharply bowing it down and under the insect can thrust the abdomen far forward until the mouth parts are reached. Owing to the general rigidity of the chitinous exoskeleton the head has very limited movements in any direction. The mouth parts can reach the fore and middle legs well up the femur, and can be used directly on these structures in the moistening and cleaning operations. Most other parts of the body must be moistened and cleaned indirectly, by employing the tarsal joints. The marvelous thing about the whole process is the thorough and practical manner in which it is all done. The katydid knows all the limitations of its various body-structures and appendages and appears to work with a complete knowledge of the degree of their extensibility in every direction, and the extent to which each leg can be employed in reaching any other part of the body.

Some parts of the body are quite inaccessible to the little katydid owing to the immobilities of its chitinous exoskeleton. No ordinary katydid has yet found it convenient to reach the basal portion of the tegmina and the musical organs situated here, or the upper portion of the pronotum. These are as inaccessible to the poor katydid as the middle of the shoulders and back are to a human. A scientist once said to me that his back had never been actually washed in twenty-five years, but simply wore itself clean with perspiration and friction of the clothes worn. A katydid's back is just as inaccessible with his rather immobile suit of chitinous armor, and no doubt he knows it too.

To my mind there is as much evidence in the katydid of an innate free will or exercise of choice in the matter of keeping clean as there is when men wash their own bodies. It makes no difference whether it be the act of a katydid or cat or man himself, the aims and functions of each are one and the same. It is done for comfort and for health. Somehow the lowly katydid, as we choose to call it, knows the sum and substance of it all, personal comfort and hygiene, as well as man himself. It is a principle of life just as much as social organization with the bees,

or family organization with the birds. Comfort and cleanliness are phases of self-preservation, and constitute a fundamental law of life. An unsocial insect as the katydid must make it an individual matter; but among the bees and men it becomes a part of the general social plan and play of an efficiently organized social life. It is here projected from the individual to the social consciousness with infinite variations. The bird has its own personal hygiene, but it has in addition a mind for the hygiene of the nest and the nestlings until they can develop this consciousness and helpfulness themselves. It is all one of the beautiful expressions of life and its supreme necessities, amounting even to an art of judgment and adjustment as in the case of the little katydid.

All insects have their specific and appropriate cleaning habits. It appears to be a universal behavior with life. The lowliest ameba would seem to have its likes and dislikes, its appreciations of comforts and discomforts, the useful and the useless in its metabolisms and behaviors. One can expect infinite variations of the theme with every species, because there are eternally different degrees of form and function, freedom and limitation. It is one of the vital principles of life, and a man could spend his entire days upon the subject, for every creature is a law unto itself. While some katydids and grasshoppers run their antennae through their mouth parts to clean them, others step upon them and draw them beneath their feet. Being a fundamental necessity of life, involving as it does the comfort and health of a creature, one can expect this behavior to present wonderful and varied manifestations, involving always the fundamentals of refined judgments and efficient movements.

It is probably no idle play of life, but one of the supreme necessities of successful existence, to keep well oiled and lubricated and groomed. The fundamental concept of comfort and health appears to be the activating motive. Sanitation, hygiene, at every turn in life, appear to have entered the consciousness of the individual and the species. Cleanliness appears to be an innate mood, as if it were well known that a clean body may thwart bacterial or fungous invasions. There are probably esthetic considerations in it as well. It would be interesting to know all the properties of the juices of the mouth parts, as a cleansing agent, as a lubricating oil, as a bactericidal wash. As we have our mosquitoes and fleas and mites to trouble us, so do all other creatures

have their parasite troubles. If we anoint ourselves with this and that to soften our skins or drive away our pests, no doubt the katydid has leanings in the same direction—in the direction of comfort and peace—as positive as our own.

The poisoning methods of man to rid his premises of such pests as the cockroach have taken advantage of the cleaning tendencies of this insect. When powdered sodium fluoride is scattered over their runs, they become more or less dusted with it, and get it upon their feet. In trying to rid themselves of this irritant dust it is transferred to their mouths and enough is ingested to prove fatal to them, even though it would never be consumed voluntarily as a food substance.

One wonders how the little katydid has so thoroughly learned all the accessibilities and inaccessibilities of its anatomy. If it has learned it by personal experience, gaining facility by repetition, then it is like men apt at learning and as intelligent as we who claim the powers of reasoning. If it is all an inherited grace, then we know nothing of life and its potentialities and evolutions. The more I know of the graces and arts of katydids, the more do I see them as creatures of good practical judgments and free will such as I would pronounce for the normal behaviors of men.

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